825

Controls on biomass:nutrient ratios in stream algae

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Table of Contents

General Introduction

Algae respond in multiple ways to changes in their resource base. Both nutrients and light intensity can influence algal growth rates, photosynthetic rates, species composition and diversity, and algal elemental composition. Although these relationships have been examined extensively in freshwater lakes, little study has considered these processes in streams.

I focus here on the influence of light and nutrients on the carbon, nitrogen, and phosphorus composition (C:N:P) of stream algae. The relative amounts of algal carbon fixation and nutrient assimilation control algal C:nutrient ratios. This C:nutrient ratio is also known as "stoichiometric yield," or the amount of biomass produced (in terms of carbon) per unit of the limiting nutrient. Thus, with a constant yield, the amount of limiting nutrient will dictate the quantity of biomass production. However, in natural systems, algal stoichiometric yield may vary by almost an order of magnitude, and therefore the amount of biomass produced per unit nutrient input will vary depending on the algal C:nutrient composition. Stoichiometric yield is thus of importance in ecosystem management when one attempts to regulate primary production. With a certain nutrient input to a system, the amount of biomass produced can vary widely, depending on the yield, or the C:nutrient composition, of the primary producers. Understanding which factors influence stoichiometric yield will therefore help in predicting responses in primary production to nutrient inputs in a system. This thesis investigates the influence of available light and nutrients on the elemental and community composition of stream algae.

In Chapter 1, I present results of an experiment in which I manipulated levels of nitrogen, phosphorus, and light intensity in four streams, and examined the response of periphyton. I looked at how the general taxonomic composition of the periphyton varied in response to changes in the resource base, and I also examined how the periphyton C:N:P responded. I found that a more severely nutrient limited system had more pronounced responses to the manipulations.

In Chapter 2, I describe results of an observational field study designed to investigate the elemental composition of both periphyton and suspended algae in streams, and how the composition varies in different light and nutrient regimes. I did not find a strong relationship between the balance of light and nutrients (light:nutrient ratio) and algal elemental composition, perhaps due to a general lack of nutrient limitation in the systems studied. I found distinct C:N:P ratios between the periphyton and suspended matter communities within the streams, and I suggest two hypotheses to account for this difference, one abiotic and the other biotic. I also tested the utility of periphyton C:N:P as an indicator of nutrient limitation status in streams, and I conclude that periphyton C:N:P is not a useful indicator of nutrient limitation in streams.

Chapter 1: Light and nutrients affect the elemental and taxonomic composition of stream periphyton

Abstract

Although the balance of available light and nutrients has been shown to influence the C:nutrient content of lake seston, few studies have considered the influence of the light:nutrient ratio on stream algae. In this study, the responses in the elemental and taxonomic composition of stream periphyton to manipulations in light, nitrogen and phosphorus were examined in four north-central Minnesota streams. I predicted that periphyton C:N and C:P would decrease as a result of higher dissolved nutrient concentrations, lower light intensity, and lower light availability relative to nutrients (light:nutrient). Nutrient diffusing artificial substrata were placed in each stream bed, and plexiglass was used to shade half of the substrata. The carbon, nitrogen and phosphorus content of the periphyton was analyzed and the algal taxonomic composition was estimated by an analysis of accessory photosynthetic pigments that served as marker pigments for green algae, diatoms, and cyanobacteria.

Primary production at two of the sites responded to shading, one stream was potentially nitrogen limited, and the fourth site was co-limited by both nitrogen and phosphorus. This N and P co-limited site was the most severely nutrient limited site out of the four, as indicated by an 860% increase in chlorophyll *a* in the treatments with addition of both N and P, compared to the control. The chlorophyll response to addition of the limiting factor at the other three sites was an order of magnitude less. The periphyton elemental and taxonomic composition at the severely limited site also

responded more strongly to the treatments than did the periphyton at the other sites. Periphyton C:N decreased in response to both the shade treatment and the phosphorus addition, and phosphorus addition increased the proportion of cyanobacteria and decreased the proportion of green algae. In the other three sites, nutrient and/or light limitation was weaker, and the periphyton did not respond to the manipulations as predicted. This experiment demonstrated that the elemental and taxonomic composition of periphyton in a strongly nutrient limited stream will respond more dynamically to changes in light and nutrient availability than will periphyton in a stream that is either not light or nutrient limited or is more weakly limited.

Introduction

Algae respond to varying amounts of available light and nutrients through shifts in both elemental composition and community composition. Algal carbon, nitrogen and phosphorus content (C:N:P) is driven by the processes of carbon fixation and nutrient assimilation, and factors that affect these two processes influence elemental composition. Sunlight provides energy for carbon fixation with light intensity affecting rates of photosynthesis. Higher light intensities can increase algal growth capacity and photosynthetic rates, leading to more carbon fixation and hence higher C:nutrient ratios (Healey 1985). Nutrient limited algae have a lower nutrient content than non-limited algae, and a higher nutrient availability can decrease the algal C:nutrient (Goldman et al. 1979). Algae growing at maximal growth rates under the absence of nutrient limitation have been shown to have lower C:nutrient ratios than algae growing at lower growth rates (Goldman et al. 1979). Algal C:N:P may also respond to the relative amounts of light

and nutrients, with higher light:nutrient ratios leading to higher algal C:nutrient (Sterner et al. 1997). In this scenario, as growth rates increase due to increasing light intensity, while nutrient availability does not increase, carbon fixation increases relative to nutrient assimilation. Sterner et al. (1997) found evidence for this "light:nutrient" hypothesis in a set of temperate freshwater lakes. These patterns would only be expected in a system that was both nutrient and light limited. If increased light intensity does not increase photosynthetic rates, then carbon fixation would not increase and algal carbon content would not change. In a system that is not nutrient limited, an increase in rates of carbon fixation would be accompanied by an increase in nutrient assimilation.

These patterns are well documented for phytoplankton in temperate lake ecosystems, but less so in benthic stream habitats (Sterner and Elser 2002). The stream benthos may differ from the environment experienced by lake phytoplankton in that boundary layers are thicker in the benthos, and water velocity in streams is generally higher than in lentic environments. Increased water velocity can increase the nutrient supply to periphyton (Stevenson and Glover 1993). There is some evidence that increased dissolved nutrient concentrations can decrease stream periphyton C:nutrient (Peterson et al. 1993, Rosemond et al. 1993, Stelzer and Lamberti 2001). Although there is a variety of evidence of light limitation in stream periphyton (Lowe et al. 1986, Hill and Harvey 1990, Steinman 1992), there is a lack of information regarding the effects of changes in light intensity on the C:N:P of stream periphyton.

In addition to affecting elemental composition, resource availability may also influence algal species composition, through resource competition (Tilman 1982). For example, nitrogen-fixing cyanobacteria are better competitors for nitrogen than are non-

fixing algae, and often become more abundant when nitrogen levels are low relative to other nutrient concentrations (e.g. Sterner 1989, Mulholland et al. 1995). Different algal classes have different light requirements for optimal growth. Diatoms (Bacillariophyceae) generally can tolerate a wide range of light intensities, while cyanobacteria demonstrate high growth and photosynthetic rates at lower light intensities (Richardson et al. 1983). Conversely, green algae (Chlorophyta) grow better at relatively higher light levels. An indication of general algal taxonomic composition can be obtained through photosynthetic pigment analysis. Various pigments, known as marker pigments, are indicative of algal taxonomic divisions and can thus be used to describe the community composition of a sample (Millie et al. 1993). Although this method has been used to characterize phytoplankton populations in estuaries (Tester et al. 1995), coastal waters (Millie et al. 1997), and freshwater habitats (Descy et al. 2000), it has rarely been used in benthic algal studies (Havens et al. 1999).

I performed experiments in four Minnesota streams with nutrient-diffusing artificial substrata in which I manipulated levels of nitrogen, phosphorus, and light, and examined the effects on the C:N:P and community composition of stream periphyton. I predicted that periphyton C:N and C:P would decrease as a result of higher dissolved nutrient concentrations, lower light intensity, and lower light availability relative to nutrients (light:nutrient). I also examined the effects of light and nutrients on the relative amounts of cyanobacteria, diatoms, and green algae in the periphyton communities.

Methods

Study sites

The four experimental sites were located in north-central Minnesota, and flow over calcareous glacial till deposited from the Des Moines Lobe of the Laurentide Ice Sheet. They were all first or second order streams in the vicinity of Itasca State Park. I selected reaches located near roads to facilitate the sampling and experimental set-up. Criteria for selection included that the streams had to be deep enough so that the racks of bottles would be covered by water (0.2 meters), but less than approximately 0.5 meters to allow the experimental set-up and maintenance.

Bear Creek was a second order stream, surrounded by mixed coniferous-deciduous forest, with alders along the banks. The substrate ranged from sand sized particles to larger cobbles. Water depth at base flow was approximately 0.2 meters, and the stream channel was 2.5 meters wide. Vegetation at Sucker Brook, also a second order stream, consisted of grasses and sedges up to one meter high immediately adjacent to the stream, and a mixed coniferous-deciduous forest approximately 30 meters away. Substrate size was on the average smaller than at Bear Creek, and aquatic macrophytes were more abundant. At base flow, the stream channel was 1.5 meters wide and approximately 0.3 meters deep. Vegetation at Nicollet Creek, a first order stream, was similar to that at Sucker Brook, the channel width was 2.3 meters, and the channel depth was 0.4 meters. La Salle Creek, a first order stream draining from La Salle Lake approximately 100 meters upstream from the experimental reach, at base flow was seven meters wide and approximately 0.5 meters deep. The substrate consisted mostly of

gravel and sand. At the experimental reach, the stream channel was bordered by dead spruce trees, with a mixed coniferous-deciduous assemblage further away from the stream.

Stream characteristics

The streams were sampled for dissolved nutrients on July 6, July 13, and July 27. Grab samples were collected in two liter plastic bottles, and filtered through pre-combusted and pre-rinsed GF/F filters into acid-washed polyethylene plastic bottles. Within 15 hours, samples were analyzed for soluble reactive phosphorus (SRP), nitrate plus nitrite-nitrogen (NO_3^-/NO_2^--N), and ammonium-nitrogen (NH_4^+-N) on an AlpKem Flow-3000 autoanalyzer. Samples for total dissolved phosphorus (TDP) and dissolved reactive silica (DRSi) were frozen at -70°C until later analysis – persulfate digestion (Wetzel and Likens 1979) followed by SRP analysis by the ascorbic acid method (APHA 1995), and the molybdosilicate method (APHA 1995), respectively.

At each site, the proportion of open canopy was estimated using a densiometer, water velocity was measured with a Global Water Flow Probe, dissolved oxygen and temperature were measured with a YSI-55 dissolved oxygen meter, pH was measured with a Corning Model pH-40 meter and conductivity was measured with a YSI Model 33 conductivity meter. Discharge was calculated from water velocity and channel width and depth measurements (Gore 1996). Vertical attenuation coefficients (Hauer and Hill 1996) were estimated using a Biospherical Instruments QSL-100 quantum sensor.

Two indices of the relative amount of light and nutrients at each site were calculated by dividing the proportion of open canopy by both the average soluble reactive

phosphorus (SRP) and the dissolved inorganic nitrogen concentrations (DIN; equal to nitrate/nitrite + ammonium-nitrogen). These values allowed me to compare the light:nutrient environments among sites.

Nutrient diffusing aritificial substrate experiment

I utilized a modified version of Matlock periphytometers (Matlock et al. 1998), a type of nutrient diffusing artificial substrate. Half-liter polycarbonate bottles were filled with nutrient-amended water and autoclaved to sterilize the medium. A nylon membrane (Whatman 0.45 μm pore size, 47 mm diameter) was then placed over the mouth of the bottle, and a glass fiber filter (Whatman GF/F, 37 mm diameter) was placed over the membrane. Caps with holes drilled into them were then screwed onto the bottles, and the bottles were placed in racks which were subsequently secured to the bottom of the stream. Water and nutrients in the bottle passively diffuse through the nylon membrane and the glass fiber filter, which serves as the artificial substrate. Algae and other microflora colonize the filter, and are exposed to nutrients in the stream water plus the nutrients from within the bottle. There were four treatments: control (no nutrients added), nitrogen-amended (+N, 2.6 mM $NaNO_3$), phosphorus-amended (+P, 0.65 mM $Na_2HPO_4 \cdot 7H_20$), and both nitrogen and phosphorus-amended (+NP, 2.6 mM $NaNO_3$, 0.65 mM $Na_2HPO_4 \cdot 7H_20$). Light level was also manipulated; half of the bottles were covered with Plexiglass which let through 27% of incoming visible light, and blocked UV light. The other half were covered with clear plexiglass that only blocked UV light. I placed 16 racks of four bottles each in each stream, with each rack containing one bottle of each of the four nutrient treatments, in a randomized order. Each rack was covered

with a piece of either the shaded or clear plexiglass. Thus, I used a split plot design, with light level as the whole plot factor and nitrogen and phosphorus as the split plot factors. Racks were paired; the filters on one rack in each pair were designated for carbon, nitrogen and phosphorus analysis, and the filters from the other rack in the pair were designated for pigment analysis.

Racks were secured to the bottom of each stream for approximately 2.5 weeks. Sites were visited every other day during which material that had been caught on the racks was removed and the top of the plexiglass was wiped clean with a sponge. The date of the end of each experiment was determined by the level of algal growth on the filters and the deterioration of the filters, most likely due to physical abrasion from the water and suspended particles in it. I wanted enough biomass in order to be able to measure the nutrient and pigment content, but I couldn't leave the bottles in the water for too long. At the end of the experiment, the racks of bottles were removed from the streams, and the filters were transported in the dark and on ice to the lab where they were frozen. Filters designated for pigment analysis were frozen at -70°C until analysis, and nutrient filters were dried in a drying oven overnight at 60°C and stored in a dessicator.

Periphyton nutrient analyses

Before the nutrient analysis, filters were cut in half. One of the halves of each filter was exposed to concentrated hydrochloric acid fumes overnight to drive off inorganic carbon, redried in a drying oven, and particulate carbon and nitrogen content was analyzed using a Perkin Elmer 2400 CHN Elemental Analyzer. The other half was digested with potassium persulfate (Wetzel and Likens 1979) and analyzed for SRP with

the ascorbic acid method (APHA 1995). Factorial analysis of variance (ANOVA) was utilized to determine the effects of light, nitrogen, and phosphorus on periphyton nutrient content. Data were transformed as necessary to meet the assumptions of normality and homogeneity of variances.

I was unable to analyze the phosphorus content of the periphyton growing on the phosphorus amended filters. Phosphate from the nutrient amended water inside of the bottles was present in the filters when they were removed from the bottles. After the experiment was completed, I tested this for both phosphate and nitrate by placing a P-amended and N-amended periphytometer in water overnight, removing the filters as I had done in the field, and analyzing the filters for total phosphorus and CHN, respectively. The two phosphorus replicates yielded 2.9 μg P and 5.6 μg P per half-filter, while the amount of phosphorus on the experiment filters ranged from 1.15 μg P to 8.81 μg P, with an average of 3.9 μg. I therefore did not use the phosphorus data from the periphyton on the P-amended bottles, but I was able to use phosphorus data from both the control and the N-amended bottles. The two nitrate test filters yielded 1.44 μg N and 2.35 μg N per half-filter. The range of nitrogen on the experiment filters was from 6.68 μg to 163.6 μg and averaged 35 μg N. Since the amount of nitrogen on the filters originating from the nutrient amendment was low relative to the total amount of nitrogen on the filters, I felt comfortable using the data. I subtracted 1.9 μg N (the average μg N from the two test filters) from the quantity of N on each filter that came from a +N or +NP bottle. I did not adjust the N levels from the control or the +P bottles.

Hypotheses regarding the effects of light:N on periphyton C:N were tested with pre-planned contrasts. I was unable to test the effects of light:P on periphyton C:P, due to

not being able to use the periphyton P data. Treatments were arranged in order of increasing light:N: shaded +N in the lowest group, shaded -N and open +N in the middle group, and open -N as having the highest light:N. I predicted that as the light:N increased, the periphyton C:N would increase as well. I tested this separately in the -P and +P treatments, since phosphorus influenced periphyton C:N in some sites and a significant N effect could be confounded with a P effect if the data were analyzed together. Therefore, for each site and P level, I performed five pre-planned pairwise contrasts, outlined in Table 5. The predicted change for each of these comparisons was positive. In all of my statistical analyses, an effect was considered significant if the *P* value was less than 0.05.

Pigment analyses

High-performance liquid chromatography (HPLC) was utilized to separate and identify pigments on the filters. Three ml of 100% acetone were added to each filter, leading to a final concentration of approximately 90% acetone due to water in the filter from the time at which it was frozen. The samples were sonicated for five seconds and then extracted for 12 to 16 hours at 4°C. After extraction, the samples were filtered through Millipore 0.2 μm filters into amber vials and injected into a Hewlett Packard model 1090 HPLC equipped with a single monomeric (Hewlett Packard ODS Hypersil; 100 x 4.6 mm, 5 m) and two polymeric (Vydac 201TP, 250 x 4.6, 5 m) reverse-phase C_{18} columns in series, a photodiode array detector, and an in-line Hewlett-Packard Model 1046A fluorometer. The column temperature was 38°C. The mobile phases and solvent flow rates utilized are described in Pinckney et al. (1996). Pigment peaks were identified

and quantified at 436 nm by comparison of retention times and absorption spectra to those of standards.

Chlorophyll *a* was used to estimate total algal abundance. The accessory pigments fucoxanthin, zeaxanthin, lutein and chlorophyll *b* were used to estimate the abundance of cyanobacteria (Cyanophyta), diatoms (Bacillariophyta), and green algae (Chlorophyta; both lutein and chlorophyll *b* are found in green algae), respectively. The contribution of each pigment to total algal abundance was estimated with linear regression (Jeffrey et al. 1999), with chlorophyll *a* as the dependent variable and the marker pigments as the predictors. The regression coefficients for each predictor are estimates of the chlorophyll *a*:marker pigment ratios. Using the amount of marker pigment in each sample, the regression coefficients were then used to calculate the proportion of chlorophyll *a* attributed to each marker pigment in that sample. By normalizing the amount of the marker pigments to chlorophyll *a*, one can compare the relative proportions of each algal group in terms of biomass, as indicated by the amount of chlorophyll *a* attributed to their marker pigments. This method assumes a constant pigment ratio within a sample group (Mackey et al. 1996). However, chorophyll *a*: accessory pigment ratios are known to vary with changes in light intensity (Descy et al. 2000). Therefore, data from each site and light treatment were run through separate regressions. For each data set, either lutein or chlorophyll *b* was used to estimate chlorophyte abundance, depending on which of the two pigments was more prevalent in the samples.

This method does not distinguish between algal groups with shared marker pigments. Fucoxanthin is found in diatoms, chrysophytes, and dinophytes; chlorophyll *b*

is common to both chlorophytes and euglenophytes; and zeaxanthin is present in both cyanobacteria and cryptophytes (Millie et al. 1993). However, chrysophytes and cryptophytes are unlikely to be found on surfaces in flowing water, as they are more common in pelagic systems.

I used multiple analysis of variance (MANOVA) to determine if the factors light, nitrogen, and phosphorus had an effect on the community composition at each of the four sites, as indicated by changes in relative proportions of the amount of chlorophyll *a* attributed to each accessory pigment. Proportions were calculated for each sample by dividing the amount of chlorophyll *a* attributed to each marker pigment by the amount of chlorophyll *a* attributed to all of the marker pigments that I used in the analysis for that site. This measure of total chlorophyll *a* was in most cases lower than the actual amount of chlorophyll *a* measured in the samples, since other accessory pigments were present that I did not use as marker pigments in my analysis, such as chlorophyll *c*, diadinoxanthin, violaxanthin, and neoxanthin. For ease of discussion, when I use the name of a pigment in this paper, it will indicate the amount of chlorophyll *a* attributed to that pigment.

Analysis of variance was used to determine which factor was limiting to primary production at each site, with chlorophyll *a* as the response variable, and light, nitrogen and phosphorus as predictor variables. A factor was considered to be limiting at $P < 0.05$. If chlorophyll *a* was significantly higher in the +NP treatment than all of the other treatments, the site was considered to be co-limited by nitrogen and phosphorus.

Results

The physical characteristics of the streams are presented in Table 1. Both Nicollet Creek and Sucker Brook had higher percent dissolved oxygen and a higher proportion of open canopy than the other two sites. Light attenuation in Bear Creek was higher than in the other sites. Dissolved nutrient concentrations varied among sites, as well as over time (Table 2). SRP levels at La Salle Creek were below the detection limit on all three sampling dates.

The relative amount of light and soluble reactive phosphorus varied among the sites by almost two orders of magnitude (Table 3). La Salle had a relatively open canopy and SRP levels below the detection limit, leading to an index of light:SRP of 2000, while the light:SRP at the other three sites ranged from 32 to 242. Light:DIN was not as variable; this index was highest in Sucker Brook (71) and relatively similar among the other three sites (27 to 39; Table 3).

Each of the four sites responded differently to the manipulations of light and nutrients. In Bear Creek, light significantly influenced chlorophyll *a* (Table 4). However, this does not mean that light limited primary production in the stream. I experimentally *decreased* light level with the shade treatment; in order to conclude that light was limiting, I would have to observe an increase in chlorophyll *a* due to an experimental *increase* in light level. A decrease in chlorophyll due to a decrease in light does not necessarily mean that additional light would increase primary production. Nonetheless, a significant light factor indicates that irradiance was either near or below the light saturation phase of the photosynthesis-irradiance (P-I) curve, and hence the system was either light limited or close to light limitation. Chlorophyll *a* in the shade

treatment was 77% lower on average than chlorophyll in the open treatment, 0.22 $\mu g/cm^2$ vs. 0.96 $\mu g/cm^2$, respectively. C:N was higher in the shade treatments, as was C:chlorophyll (Table 4). The light:nutrient hypothesis was not supported in Bear Creek. In the -P treatments, none of the pre-planned contrasts were significant. In the +P treatments, two of the five pre-planned contrasts were significant, although the effects were negative, opposite of the predicted direction (Table 5).

Regression analysis was used to estimate the chlorophyll *a*:marker pigment ratios from the regression coefficients. The regression coefficients for each marker pigment varied between the two regressions performed for each site, using the data from the open and shaded treatments separately (Table 6). At Bear Creek, the linear regression of the pigment data from the shaded treatments yielded a higher R^2 than the regression from the open treatments (0.91 vs. 0.62, respectively; Table 6). Neither chlorophyll *b*, lutein, or zeaxanthin were present on the filters from the shaded treatment, and chlorophyll *b* was present in only four of the sixteen open treatment samples. This variability led to the low R^2 in the open treatments group. Most of the chlorophyll *a* in the Bear Creek experiment was attributed to fucoxanthin (Figure 1A), suggesting that diatoms dominated. Chlorophyll *b*, indicative of green algae, was present in all treatments except for the shaded treatments. Neither nitrogen, phosphorus nor light significantly affected the proportions of chlorophyll *b* and fucoxanthin in the site (Table 7).

At Sucker Brook, chlorophyll *a* was potentially limited by nitrogen ($P = 0.06$; Table 4); the added nitrogen increased chlorophyll *a* by 82% (1.04 $\mu g/cm^2$ vs 1.90 $\mu g/cm^2$). Carbon was lower in the shaded treatments, and P had a significant negative effect on periphyton C:N. Figure 2 illustrates the significant light x P interaction term

and reveals that the negative P effect on C:N existed in the shaded treatment but not in the open treatment. Nitrogen had a negative effect on periphyton C:chlorophyll *a*. With regard to the light:nutrient hypothesis, none of the pre-planned contrasts in the -P treatments were significant, while one contrast in the +P treatments was significant, although in the direction opposite of that predicted (Table 5).

R^2 from the regressions for the Sucker Brook data were both 0.99 (Table 6). Fucoxanthin, chlorophyll *b*, and zeaxanthin were all present, indicating the existence of communities of diatoms, green algae, and cyanobacteria, respectively (Figure 1B). None of the terms in the MANOVA testing the effects on community composition were significant (Table 7).

Light influenced chlorophyll *a* at Nicollet Creek in this experiment (Table 4). Chlorophyll *a* decreased by 44% in the shade treatment (0.14 $\mu g/cm^2$), relative to the open treatment (0.25 $\mu g/cm^2$). None of the contrasts testing the light:nutrient hypotheses were significant in this site (Table 5).

The pigment analysis regressions at Nicollet Creek accounted for most of the variability in chlorophyll *a* (Table 6). Primary producers were dominated by diatoms and green algae, as indicated by the presence of fucoxanthin and lutein, respectively (Figure 1C). Light significantly influenced the proportions of these groups (Table 7). The shade treatment increased the proportion of fucoxanthin (diatoms), and decreased the proportion of lutein (green algae; Scheffé post-hoc comparison, df = 23, $P = 0.03$).

At La Salle Creek, nitrogen and phosphorus co-limited chlorophyll *a*. The positive effect that nitrogen had on chlorophyll *a* only occurred in the +P treatments (Table 4, Figure 3a). In the -P treatment, adding N did not increase chlorophyll *a*

(Scheffé post-hoc comparison, df = 23, $P = 1.0$), whereas adding both nutrients greatly enhanced primary production (df = 23, $P < 0.00001$). Chlorophyll *a* in the +NP treatments (4.94 $\mu g/cm^2$) was 860% greater than the average chlorophyll *a* of the control, +N, and +P treatments combined (0.51 $\mu g/cm^2$). N and P had a similar effect on μg C; P increased the amount of C on the filters, but only in the presence of N (Figure 3b).

Both shade and phosphorus significantly decreased periphyton C:N in La Salle (Table 4), and all of the interaction coefficients were significant. The light x N x P interaction (Figure 4) illustrates that light had an effect only when both N and P were added, and phosphorus had an effect only in the shaded treatment when N was added. In the -P treatments, two of the five pre-planned contrasts regarding the light:nutrient hypotheses were significant and positive, and three of the five contrasts in the +P treatments were also significant and positive (Table 5).

The regression equation for the shaded treatment at La Salle explained more of the variability in chlorophyll *a* than did the equation for the open treatment (Table 6). The algal community was dominated by diatoms, green algae, and cyanobacteria, as indicated by the presence of fucoxanthin, lutein, and zeaxanthin, respectively, in the pigment analysis (Figure 1d). Both phosphorus and light significantly influenced the algal community composition in the experiment (Table 7). Phosphorus addition decreased the proportion of lutein, or green algae (Scheffé post-hoc comparison, df = 23, $P = 0.0003$) and increased the proportion of zeaxanthin, or cyanobacteria (Scheffé post-hoc comparison, df = 23, $P = 0.001$). The shade treatment had the opposite effect; lower light treatments had relatively more lutein (Scheffé post-hoc comparison, df = 23, $P = 0.006$) and relatively less zeaxanthin (Scheffé post-hoc comparison, df = 23, $P = 0.01$).

Discussion

These four streams responded differently to manipulations in light, nitrogen, and phosphorus. Primary production, as measured by algal biomass, at two of the streams was reduced by shading, one stream was potentially nitrogen limited, and the fourth stream was co-limited by both nitrogen and phosphorus (Table 4). The predicted changes in periphyton C:N, based on the light:nutrient hypothesis, were observed only in La Salle Creek, which is the N and P co-limited site and is located directly downstream from a lake. This site was the most imbalanced of the sites in terms of the relative amounts of light and phosphorus (Table 3), and also the most severely limited site, as measured by the relative increase in primary production when the limiting nutrient was supplied in excess. Chlorophyll *a* increased by over 800% in the +NP treatment, compared to changes in the other sites of only 44 to 82% in response to manipulation of the limiting factor. It has been demonstrated that as nutrient limitation becomes more severe, algal C:nutrient increases (Goldman et al. 1979). Periphyton C:N in La Salle decreased in response to both the shade treatment and phosphorus addition. The P effect on C:N occurred only in the shaded +N treatment, and the light effect occurred only in the +NP treatment (Figure 4). The dissolved nitrogen and phosphorus levels were so low in this stream (Table 2) that there were few significant changes in either the quantity (Figure 3) or the elemental composition (Figure 4) of the periphyton without both nitrogen and phosphorus additions. This site was located approximately 100 meters downstream from La Salle Lake, and most likely drained low nutrient epilimnetic water. Thus, the location of a stream reach with respect to lakes in its drainage basin can influence ambient

nutrient levels and in turn affect the response of periphyton after either nutrient additions or alterations in the light environment.

Changes in periphyton community composition in response to the treatments were also more pronounced in the severely limited stream (Figure 1). Phosphorus addition in La Salle Creek increased the proportion of cyanobacteria and decreased the proportion of green algae. Dissolved N and P levels in this stream were low enough that the N-fixing ability of cyanobacteria only gave them an advantage over other species in the +P treatment. Although N did not have a significant effect on community composition in La Salle, qualitatively there was a greater proportion of cyanobacteria in all of the -N treatments, compared to the +N treatments. Likewise, cyanobacteria in a nitrogen-limited desert stream were found to be more prevalent under ambient nutrient conditions than in N amended treatments (Peterson and Grimm 1992).

There were more cyanobacteria and fewer green algae in the open treatment relative to the shaded treatment in La Salle. This is contrary to findings of Richardson et al. (1983). In a literature review, they found that green algae generally grow better at high light intensities, and cyanobacteria at lower light intensities. In my experiment, species differences in growth and photosynthesis within the divisions with respect to light (Richardson et al. 1983) might have caused high variation in the algal responses to the shade treatment and could have affected the average response to light in each division. Also, photoinhibition could have been a factor influencing the algal community composition. Both UV light and visible light can induce photoinhibition in algae (Smith et al. 1980), and UV light in my experiments was blocked in both treatments by the plexiglass coverings on the racks of bottles. Cyanobacteria thus may have experienced

less photoinhibition on the covered artificial substrates than on unshaded natural substrates. The pattern at Nicollet Creek was the opposite to that at La Salle. Green algae were less common, and diatoms more common, in the shade treatment (Figure 1C). There were no significant differences in community composition at Bear Creek and Sucker Brook (Figure 1A,B). Periphyton at Bear Creek, Sucker Brook, and Nicollet Creek, in addition to not showing many responses in community composition, did not differ much in nutrient composition (Table 4). This is partly due to the fact that I was not able to use all of the periphyton C:P data in my analysis and could not fully test my hypotheses. However, I would not expect a strong response in periphyton C:P in a stream which was not phosphorus limited, and the only stream that showed signs of phosphorus limitation, with respect to chlorophyll *a*, was La Salle. Due to the severe co-limitation by both N and P at this site, I was able to detect a response in periphyton C:N to the available light and nutrients.

The regression method of determining the proportion of chlorophyll *a* attributed to marker pigments has been criticized for the assumption that the marker pigment:chlorophyll *a* ratio is constant within groups (Havens et al. 1999). Accessory pigment:chlorophyll *a* ratios often vary with light intensity (Descy et al. 2000). However, I analyzed my data in groups of similar light intensity; each group consisted of a light treatment from a single site. Within a site, canopy cover and stream depth did not widely vary, and therefore light intensity at the location of the artificial substrates in each analysis group did not widely vary either. Although some authors suggest that accessory pigment:chlorophyll *a* ratios also vary with nutrient availability (Descy et al. 2000), others have found that certain accessory pigments covary with chlorophyll *a* under

different light and nutrient conditions, and therefore accessory pigment:chlorophyll *a* ratios stay relatively constant (Goericke and Montoya 1998). In my experiment, I was unable to analyze my data grouped by nutrient treatment, as the groups would have been too small to reliably estimate regression coefficients.

This experiment demonstrated that changes in periphyton elemental composition on artificial substrates in a strongly nutrient limited site were as predicted in response to manipulations in light and nutrients. In the other three sites, however, nutrient and/or light limitation was weaker, and the periphyton did not respond to the manipulations in a manner predicted by stoichiometric theory. General periphyton taxonomic composition also changed more in the severely limited site. Resources in the other sites were more balanced and thus changes in resource availability did not lead to many noticeable differences in the periphyton community.

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Table 1. Average values of water temperature, dissolved oxygen, conductivity, pH, discharge, light extinction coefficient, and proportion of open canopy of the four stream reaches.

	Water temp (°C)	% Dissolved oxygen	Conductivity (µmhos)	pH	Discharge (m^3/s)	Light extinction coefficient	Proportion open canopy
Bear Creek	16	77	440	7.3	0.25	3.3	60
Sucker Brook	22	127	360	8.0	0.15	1.5	100
Nicollet Creek	21	122	420	7.8	0.21	1.7	97
La Salle Creek	22	86	350	7.8	0.16	1.3	80

Table 2. Mean (and standard error) concentrations of soluble reactive phosphorus (SRP), nitrate + nitrite-nitrogen (NO_3^- + NO_2^--N), ammonium-nitrogen (NH_4^+-N), and dissolved reactive silica (DRSi) of stream water grab samples. Ammonium data from July 27 are missing due to methodological problems. SRP detection limit is 0.04μM.

		SRP (μM)	*NO_3^-/ NO_2^--N (μM)*	*NH_4^+-N (μM)*	*DRSi (mM)*
Bear	*July 6*	2.16 (0.0087)	0.48 (0.012)	0.37 (0.034)	0.32 (0.0038)
	July 13	1.9 (0.0034)	2.4 (0.035)	1.1 (0.017)	0.36 (0.024)
	July 27	1.6 (0.016)	1.5 (.0060)		0.41 (0.0084)
Sucker	*July 6*	0.48 (0.0071)	0.80 (0.0027)	0.73 (0.015)	0.14 (0.0038)
	July 13	0.29 (0.0016)	0.48 (0.0062)	0.48 (0.0024)	0.18 (0.0098)
	July 27	0.46 (0.00099)	1.1 (0.0010)		0.17 (0.0011)
Nicollet	*July 6*	0.81 (0.0072)	1.5 (0.0093)	0.99 (0.014)	0.24 (0.013)
	July 13	0.84 (0.013)	1.6 (0.027)	0.61 (0.043)	0.29 (0.015)
	July 27	0.74 (0.0036)	1.9 (0.00043)		0.27 (0.0062)
La Salle	*July 6*	below detection limit	0.24 (0.035)	2.8 (0.071)	0.23 (0.010)
	July 13	"	0.24 (0.031)	1.7 (0.040)	0.26 (0.0067)
	July 27	"	0.34 (0.075)		0.23 (0.0095)

Table 3. The relative amount of light and dissolved nutrients at each site. "Light" is the proportion of open canopy, and DIN (dissolved inorganic nitrogen) represents the sum of the mean values of nitrate + nitrite-nitrogen and ammonium-nitrogen at each site. La Salle light:SRP was calculated using the SRP detection limit of 0.04 μM.

	light:SRP	light:DIN
Bear	32	27
Sucker	242	71
Nicollet	122	39
La Salle	2000	32

Table 4. F statistic, *P*-value, and direction (+/-) of change from ANOVAs at each site, with chlorophyll *a* (Chl *a*), carbon (C), C:N, C:P, N:P, and C:chlorophyll *a* as the dependent variables. L = light, N = nitrogen, P = phosphorus. Numbers in the df (degrees of freedom) row are the df_{effect}, df_{error} for each ANOVA. *P*-values < 0.05 are marked with an *. All effects for the C:P and N:P models were not able to be tested due to missing periphyton phosphorus data (see text). Continued on next page.

Treatment	*Chl a*		*C*		*C:N*		*C:P*		*N:P*		*C:Chl*	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Bear, df	1,20		1,16		1,16		1,8		1,8		1,4	
L	9.99	0.00 +*	0.36	0.56	11.85	0.00 -*	0.01	0.93	1.61	0.24	40.98	0.00 -*
N	0.04	0.84	1.44	0.25	0.04	0.85	0.45	0.52	1.06	0.33	4.08	0.11
P	0.40	0.53	0.22	0.64	1.80	0.20					2.06	0.22
L x N	0.01	0.91	0.05	0.82	0.02	0.90	0.03	0.87	0.02	0.90		
L x P	0.00	0.98	0.30	0.59	0.02	0.88						
N x P	0.95	0.34	0.12	0.74	0.07	0.80						
L x N x P	0.05	0.83	0.00	1.00	0.67	0.43						
Sucker, df	1,19		1,16		1,16		1,8		1,8		1,4	
L	1.20	0.29	9.38	0.01 +*	3.34	0.09	0.54	0.48	0.23	0.65	0.22	0.66
N	4.12	0.06	0.00	0.99	0.00	0.98	1.17	0.31	2.42	0.16	12.95	0.02 -*
P	0.11	0.74	0.71	0.41	4.95	0.04 -*					0.03	0.87
L x N	0.00	0.97	0.06	0.81	3.09	0.10	0.55	0.48	1.32	0.28		
L x P	0.46	0.51	0.32	0.58	5.29	0.04 *						
N x P	1.98	0.18	2.41	0.14	3.12	0.10						
L x N x P	0.92	0.35	0.80	0.38	1.88	0.19						

Table 4. Continued from previous page.

Treatment	*Chl a*		*C*		*C:N*		*C:P*		*N:P*		*C:Chl*	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Nicollet, df	1,23		1,16		1,16		1,8		1,8		1,4	
L	6.13	0.02 +*	0.34	0.57	0.07	0.80	0.63	0.45	0.95	0.36	1.54	0.28
N	0.45	0.51	0.87	0.36	0.11	0.75	0.05	0.82	0.01	0.94	0.00	0.97
P	0.01	0.93	0.19	0.67	0.32	0.58					0.12	0.74
L x N	0.37	0.55	2.46	0.14	0.13	0.72	1.35	0.28	0.99	0.35		
L x P	0.00	0.98	0.57	0.46	0.23	0.64						
N x P	0.76	0.39	3.47	0.08	0.00	0.98						
L x N x P	0.54	0.47	0.05	0.83	0.01	0.92						
La Salle, df	1,23		1,16		1,16		1,8		1,8		1,4	
L	0.75	0.39	1.80	0.20	40.03	0.00 +*	0.52	0.49	2.90	0.13	0.02	0.89
N	27.45	0.00 +*	2.66	0.12	0.44	0.52	1.30	0.29	3.10	0.12	4.73	0.10
P	114.63	0.00 +*	36.26	0.00 +*	11.26	0.00 -*					14.98	0.02 -*
L x N	0.89	0.35	2.25	0.15	5.44	0.03 *	0.01	0.93	0.07	0.79		
L x P	0.04	0.85	0.84	0.37	4.59	0.05 *						
N x P	31.38	0.00 *	16.81	0.00 *	7.63	0.01 *						
L x N x P	0.03	0.87	0.54	0.47	6.12	0.02 *						

Table 5. *P* values and direction of change (+/-) for those *P* values < 0.10, from pre-planned contrasts of light:nutrient hypotheses, with regards to periphyton C:N. All predicted directions of change are +.

	from treatment	to treatment	Bear	Sucker	Nicollet	La Salle
-P treatments:	shaded, +N	shaded, -N	0.65	0.57	0.96	0.23
	shaded, +N	open, +N	0.13	0.081	0.62	0.05 +
	shaded, +N	open, -N	0.16	0.32	0.87	0.26
	shaded -N	open, -N	0.31	0.14	0.91	0.04 +
	open, +N	open, -N	0.92	0.38	0.74	0.28
+P treatments:	shaded, +N	shaded, -N	0.53	0.020 -	0.98	< 0.01 +
	shaded, +N	open, +N	0.12	0.16	0.98	< 0.001 +
	shaded, +N	open, -N	0.05 -	0.46	0.75	< 0.001 +
	shaded -N	open, -N	0.02 -	0.07 +	0.77	0.14
	open, +N	open, -N	0.58	0.45	0.73	0.56

Table 6. Regression equations and R^2 from each site and light treatment used to calculate the contribution of each marker pigment to total chlorophyll *a* in each sample. Chl = chlorophyll, fuco = fucoxanthin, zeax = zeaxanthin

Site	*Regression equation*	R^2
Bear open	log chl *a* = (0.015)fuco + (0.38)chl *b*	0.62
Bear shaded	log chl *a* = (0.080)fuco	0.91
Sucker open	chl *a* = (0.43)fuco + (2.11)chl b + (33.2)zeax	0.99
Sucker shaded	chl *a* = (0.37)fuco + (4.56)chl b + (11.3)zeax	0.99
Nicollet open	chl *a* = (0.40)fuco + (16.9)lutein	0.99
Nicollet shaded	chl *a* = (0.38)fuco + (10.1)lutein	1.00
La Salle open	log chl *a* = (0.0065)fuco + (-0.76)lutein + (1.98)zeax	0.82
La Salle shaded	log chl *a* = (0.010)fuco + (-0.60)lutein + (2.35)zeax	0.93

Table 7. MANOVA results of effects of nitrogen (N), phosphorus (P) and light (L) on periphyton community composition. Dependent variables for analysis at each site: Bear Creek – proportion fucoxanthin and chlorophyll *b*; Sucker Brook – proportion fucoxanthin, lutein, and zeaxanthin; Nicollet Creek – proportion fucoxanthin and lutein; La Salle Creek – proportion fucoxanthin, lutein and zeaxanthin. Degrees of freedom for each effect are identical within site (df_{Bear} = 1, 29; df_{Sucker} = 2, 18; $df_{Nicollet}$ = 1, 23; $df_{La\ Salle}$ = 2, 22).

	Bear		***Sucker***		***Nicollet***			***La Salle***		
Effect	*F*	*P*	*F*	*P*	*F*	*P*		*F*	*P*	
N	0.83	0.45	0.61	0.56	0.00	0.96		1.67	0.21	
P	0.05	0.96	0.04	0.96	2.22	0.15		19.94	< 0.0001	*
L	2.11	0.15	1.57	0.23	5.75	0.02	*	10.70	< 0.001	*
N x P	0.14	0.87	0.89	0.43	0.45	0.51		3.80	0.04	*
N x L	0.83	0.45	0.21	0.81	0.54	0.47		2.35	0.12	
P x L	0.05	0.95	0.60	0.56	0.75	0.40		2.58	0.10	
N x P x L	0.14	0.87	1.27	0.31	2.52	0.13		0.79	0.47	

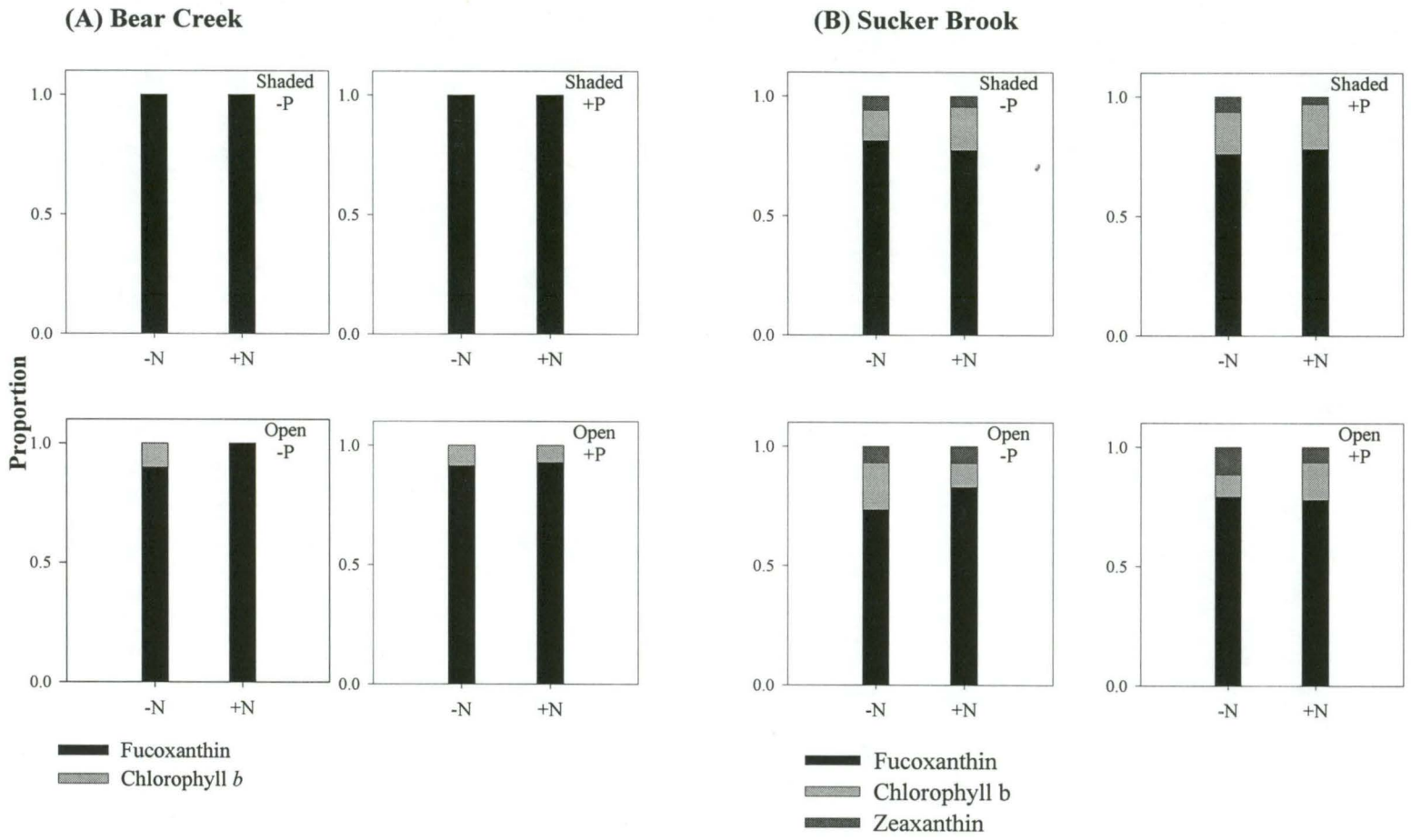

Figure 1. Proportions of chlorophyll *a* attributed to individual marker pigments at (A) Bear Creek, (B) Sucker Brook, (C) Nicollet Creek, and (D) La Salle Creek. (C and D on next page.)

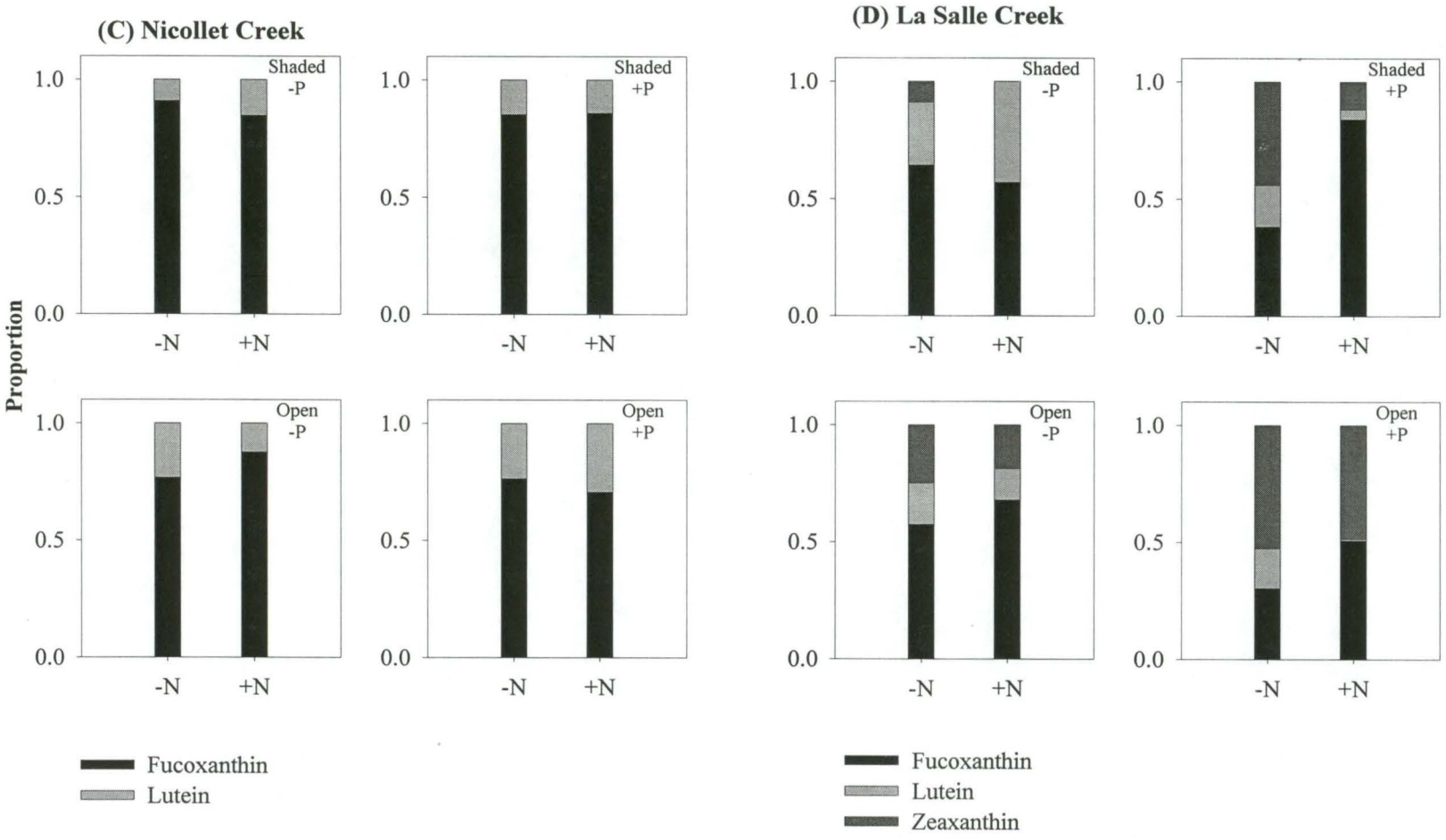

Figure 1. Proportions of marker pigments at (C) Nicollet Creek, and (D) La Salle Creek, continued from previous page.

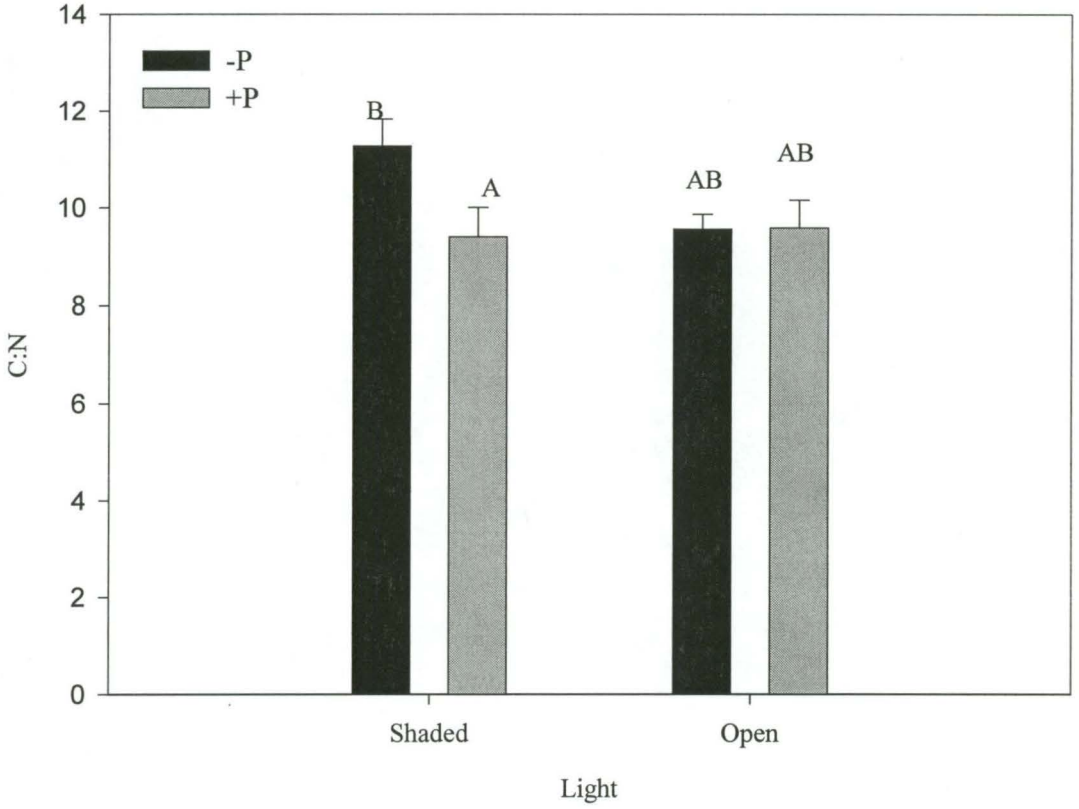

Figure 2: Periphyton C:N means +/- SE at Sucker Brook (Light x phosphorus interaction). Letters indicate groups whose means are not statistically different, Scheffe post-hoc comparison, $P = 0.05$.

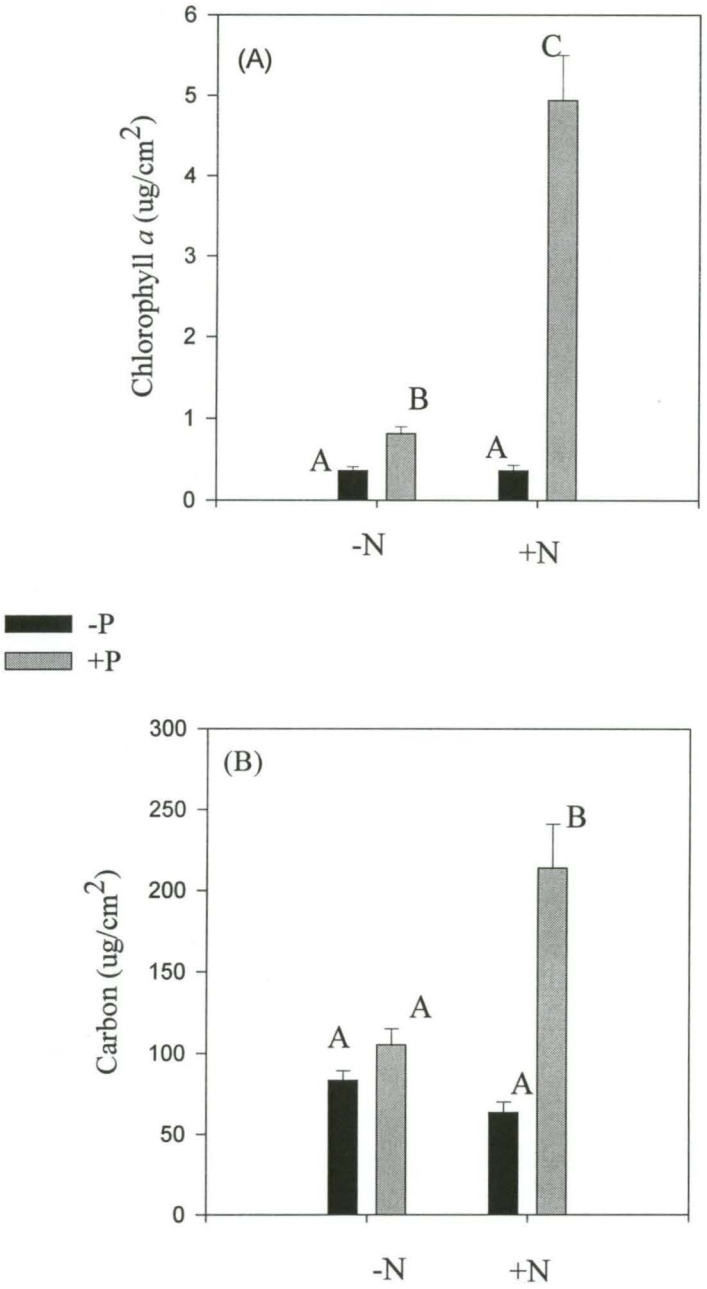

Figure 3: Periphyton (A) chlorophyll *a* and (B) carbon means +/- SE at La Salle Creek (nitrogen x phosphorus interaction). Letters indicate groups whose means are not statistically different, Scheffe post-hoc comparison, $P = 0.05$.

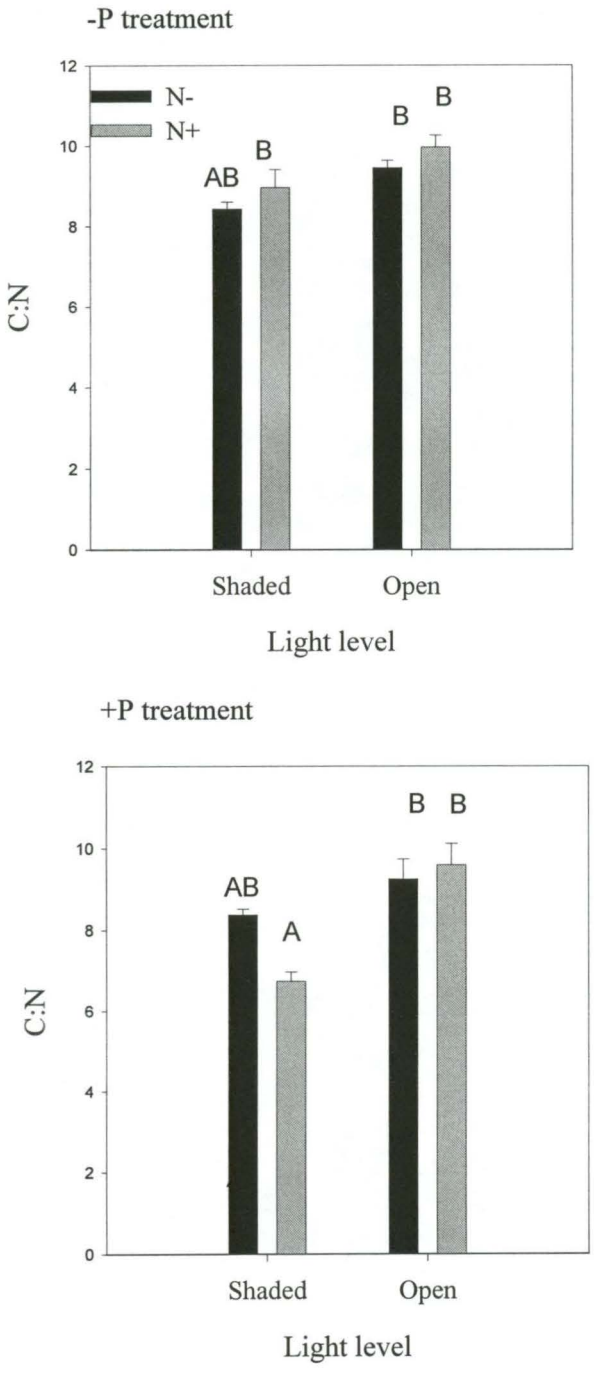

Figure 4: Periphyton C:N means +/- SE, by light, nitrogen level, and phosphorus level. Letters indicate groups whose means are not statistically different, Scheffe post-hoc comparison, $P = 0.05$.

Chapter 2: The role of physical characteristics and nutrient limitation in stream periphyton and suspended matter nutrient content

Abstract

While algal elemental composition has been extensively studied in oceans and freshwater lakes, there is a lack of information regarding the natural variation and potential predictors of the C:N:P of stream algae. In this study, the C:N:P of periphyton and suspended matter was characterized for 33 Minnesota stream reaches. Nutrient limitation experiments were performed in a subset of the streams in order to investigate the relationship between algal nutrient content and nutrient limitation. There was not a clear response of periphyton C:N:P to light availability or to the balance of light and nutrients, possibly due to a lack of severe nutrient limitation at many of the sites. SRP was a significant predictor of suspended matter C:P. The suspended matter contained more phosphorus and less nitrogen relative to the periphyton, either due to more inorganic phosphorus in the suspended matter, or due to stronger phosphorus limitation of the periphyton within the boundary layer, relative to the suspended matter. Additionally, the accuracy of using stream periphyton C:N:P to indicate algal nutrient limitation status on an instantaneous basis was tested, and was not found to be reliable.

Introduction

Studies on the impact of light and nutrients on primary productivity in streams have demonstrated that stream periphyton may be light limited (Lowe et al. 1986, Hill and Harvey 1990, Steinman 1992), nutrient limited (Tate 1990, Peterson et al. 1993,

Kutka and Richards 1997, Francoeur et al. 1999, Wold and Hershey 1999), or in other cases either light or nutrients may limit stream primary production depending on local conditions (Triska et al. 1983, Bothwell 1988, Hill and Knight 1988, Rosemond 1993, Hill et al. 1995). Light (Lowe et al. 1986, Steinman 1992, Rosemond 1994) and nutrients (Kutka and Richards 1997) have also been shown to affect periphyton community composition. Despite this body of information, little study has considered the effects of light and nutrients on periphyton elemental composition.

The stoichiometry of the conversion of inorganic nutrients and energy into biomass dictates the nutrient composition of primary producers at the base of the food web. Primary producers take up nutrients from their surrounding environment and utilize energy, usually in the form of light, to fix carbon. These two processes of carbon fixation and nutrient assimilation may be uncoupled, so that the relative amount of carbon fixed and nutrients assimilated can vary, causing differences in biomass carbon:nitrogen:phosphorus (C:N:P) ratios. Redfield (1958) noted that oceanic seston C:N:P remains relatively constant at 106:16:1. This ratio became known as the "Redfield ratio," and has been a starting point from which scientists have compared the algal C:N:P of other aquatic systems (e.g., Hecky et al. 1993).

In lake seston, C:N:P ratios have been shown to vary widely, influenced by available nutrients (Goldman et al. 1979, Healey and Hendzel 1980), light (Healey 1985), growth rate (Goldman et al. 1979, Sterner 1995), and species composition (Healey and Hendzel 1979). In addition to light and nutrients individually affecting algal elemental composition, the *relative* amount of available light to nutrients is a good predictor of lake seston C:P. Sterner and colleagues (1997) presented this light:nutrient hypothesis and

found supportive evidence in a set of temperate lakes. In a nutrient-limited system, higher light intensity can increase algal growth capacity, but if the amount of available nutrients does not increase as well, algal biomass will have a lower nutrient content and nutrient limitation will become more severe. Changing either the light environment or the nutrient availability therefore could affect the nutrient composition of the primary producers. These differences in elemental composition have implications for other trophic levels. Low nutrient phytoplankton may serve as poor food quality for consumers, and consumer growth rate may vary depending on food quality, even when food quantity (measured as carbon) is held constant (Sterner 1993). Threshold food (seston) C:P and C:N ratios were determined for two cladocerans (*Daphnia galeata* and *Bosmina longirostris*) by Urabe and Watanabe (1992), above which net zooplankton production was either P or N limited. The nutrient composition of lake seston is often at or above these threshold values, suggesting that in natural communities, these two cladocerans may be either N or P limited. Urabe and Sterner (1996) demonstrated that the balance of light intensity and nutrient availability in laboratory semibatch cultures influenced both algal and herbivore growth rates. The authors concluded that patterns of resource availability can influence herbivore growth rates, mediated by the influence of resource availability on algal abundance and chemical composition. Stelzer and Lamberti (in press) found evidence for this same phenomenon in streams. Snail growth rate was influenced by periphyton phosphorus content, which was in turn influenced by dissolved phosphorus.

Few studies have considered the controlling factors on the elemental composition of stream algae. A few studies have measured stream periphyton C:N:P ratios in

response to manipulations of light, nutrient levels, or grazers. Peterson et al. (1993) found that epilithon C:P decreased after phosphorus fertilization of a river. After fertilizing artificial channels with phosphorus and nitrogen, Stelzer and Lamberti (2001) found that periphyton C:P decreased, but C:N did not change in response to lower DIN:SRP. However, periphyton C:P and C:N did respond to changes in total nutrient concentrations. Rosemond (1993) manipulated irradiance, nutrients and herbivores in stream-side flow-through channels, and found that increased nutrients led to higher percent phosphorus and percent nitrogen of the periphyton. Additionally, grazing increased periphyton %C, %P, and %N, and reduced the C:N.

These various studies have investigated the effects of light, nutrient, and grazer manipulations on stream periphyton stoichiometry. However, there is a lack of information regarding the natural variation and potential predictors of the elemental composition of stream periphyton. Further, the elemental composition of suspended matter in streams has rarely been quantified (for an exception, see Hecky et al. 1993). Understanding this natural variation in stream algal stoichiometry will aid in further study aimed to investigate the influence of algal elemental composition on higher trophic levels. Benthic algal production has been shown to be a significant energy source in aquatic systems up to the highest trophic levels (Hecky and Hesslein 1995). Changes in periphyton nutrient content might influence growth rates of grazers in a similar manner as lake seston food quality affects consumer growth rate (Sterner 1993). Elucidating these relationships between periphyton nutrient composition and grazer productivity in streams can lead us to a broader understanding of patterns of energy and nutrient transfer through food webs.

Inherent differences between streams and lakes might lead to different patterns in elemental composition of both primary producers and consumers in these two types of ecosystems. Characteristics such as water velocity, boundary layers, canopy cover, and the biotic community of the stream environment are quite different from those of lakes. These factors could influence stream algal elemental composition through affecting the processes of carbon fixation and nutrient assimilation. For example, flowing water can increase the nutrient supply to attached algae (Stevenson and Glover 1993), which might in turn increase algal nutrient content. In addition to the differences between streams and lakes, algae within streams may experience strikingly different physical environments. The immediate environment of the attached community differs from that of the suspended community in terms of boundary layers (Hecky and Hesslein 1995) and rates of nutrient delivery (Stevenson and Glover 1993). These differences might lead to different patterns of C:N:P between stream suspended matter and periphyton.

In addition to the utility of algal C:N:P in accounting for variations in consumer growth rates, algal C:N:P has been used as an indicator of nutrient limitation in lakes, based on the assumption that there exists an "optimal" algal C:N:P for non nutrient-limited algae that are growing at near-optimal growth rates (Hecky et al. 1993). Phosphorus deficiency has been inferred from high seston C:P and N:P ratios, and likewise, N deficiency has been inferred from high C:N ratios (Healey and Hendzel 1980). One concern regarding the use of seston C:N:P as an indicator of nutrient limitation is the existence of detritus and other non-algal components of seston that have a different C:N:P composition than algae (Hecky et al. 1993). However, it has been suggested that this detrital interference is minimal in lakes with a long residence time, as

these types of lakes have low inputs of terrestrial and atmospheric particulate material (Hecky et al. 1993). Biomass C:N:P in relation to nutrient limitation has also been examined in seagrasses in coastal areas (Fourqurean et al. 1992) and in terrestrial vegetation (Verhoeven et al. 1996). The utilization of algal C:N:P in these communities to suggest nutrient limitation has been explored, but little study has considered this same technique with regards to periphyton C:N:P. Two recent studies have attempted to determine the optimal C:N:P of periphyton in order to be able to evaluate the nutrient status of freshwater benthic algae (Kahlert 1998, Hillebrand and Sommer 1999), but this method has not been tested in a variety of benthic habitats.

One of the objectives of this study was to characterize the nutrient composition of stream periphyton and suspended matter in a large set of streams, and determine which factors could best predict it. I investigated how the carbon, nitrogen and phosphorus content of stream algae compares to algae in other aquatic environments. I chose to survey a set of streams with varying amounts of available light and nutrients in order to capture as wide a range as possible in these variables. I complemented the survey data with nutrient limitation experiments in a subset of the streams in order to investigate the relationship between algal nutrient content and nutrient limitation. By combining the data from the field survey with the nutrient limitation experiments, I was able to test the accuracy of using stream periphyton C:N:P to indicate algal nutrient limitation status.

Methods

Study sites

Twenty-six Minnesota first through third order streams were selected for this study – eleven near Duluth in north-eastern (NE) Minnesota, nine in north-central (NC) Minnesota, four in Carver County in southern Minnesota, and two in the metro area of Minneapolis/St. Paul (Figure 1, Table 1). In addition to their proximity to a field station or laboratory, these four regions were chosen in order to include streams of different underlying geology and human disturbance. I based individual site selection on several criteria: the existence of rocks for collection of periphyton samples, water level no deeper than 0.70 meters so that I could obtain rocks, and proximity to a road for access purposes. Selected streams in the south were all in close proximity to corn and soybean agricultural fields, and they flow over calcareous glacial till deposited from the Des Moines Lobe of the Laurentide Ice Sheet. Streams in north-central Minnesota were neither urban nor in direct proximity to agricultural fields, and they flow over similar calcareous glacial till as the southern sites. Some of the north-east streams were located in urban and suburban areas and others were further away from direct human influence. These streams flow over Superior Lobe glacial till, material from the Canadian Shield that is low in carbonates. Both "metro" streams were urban-influenced, and they flow over material that is a mixture of the two different types of glacial till represented here.

At each of seven of the 26 selected streams, two stream reaches were sampled, usually within 50 meters of one another. One of the sites in the pair had an open canopy, and the other a closed canopy, leading to contrasting light levels at the two sites. This allowed me to compare two sites with similar nutrient regimes, yet different light levels,

and thus different available light:nutrient ratios. Only one site was sampled at each of the remaining 20 streams, leading to a total of 33 reaches.

Field sampling

Each site was sampled once during the summer. Although this sampling regime did not provide information regarding variability within a site over time, it supplied a snapshot of how current dissolved nutrient and light conditions are related to periphyton elemental composition. I did not sample streams during or within one day after a heavy rainstorm, decreasing the likelihood that dissolved nutrient concentrations were highly altered due to a rain event. At each site, percent canopy cover was estimated using a densiometer, water velocity was measured with a Global Water Flow Probe, dissolved oxygen and temperature were measured with a YSI-55 dissolved oxygen meter, and pH was measured with a Corning Model pH-40 meter. Discharge was calculated from water velocity and channel width and depth measurements (Gore 1996).

For periphyton analysis, I selected eight rocks from each stream. I chose rocks that were relatively flat on top in order to facilitate subsequent periphyton scraping. Additionally, the top surface area of each rock had to be big enough to accommodate a photographic slide mount that was used to delineate a fixed surface area. Rocks were placed in small plastic bags filled with stream water, and transported to the lab in the dark and on ice. The plastic bags were small enough such that the rocks did not move around within the bag, and I was therefore able to maintain the direction of the rock with respect to the bag and know which side had been facing up in the stream. A photographic plastic slide mount was placed over the top of each rock, and, using a toothbrush and water, only

the surface area exposed by the mount was scraped into a beaker. Sub-samples of this resulting slurry were filtered through precombusted GF/F glass fiber filters. Filters for chlorophyll analysis were immediately frozen at -20°C, and filters for carbon, nitrogen, and phosphorus analysis were dried for 24 hours in a drying oven at 60°C and then stored in a desiccator. For the suspended matter analyses, grab water samples were collected in plastic bottles, filtered through pre-combusted GF/Fs and preserved in the same manner as the periphyton sample filters.

All elemental ratios presented are on a mole:mole basis. At each site, eight rocks and two suspended matter grab samples were obtained, and two replicates of each sample were used for the chemical analyses. Periphyton and suspended matter C:N:P site means were calculated by averaging the sample C:N:P means (rock or grab sample, respectively) from each site.

Analyses for soluble reactive phosphorus (SRP), nitrate plus nitrite nitrogen (NO_3^- /NO_2^--N), and ammonium-nitrogen (NH_4^+-N) were performed on grab water samples filtered through pre-rinsed and pre-combusted GF/Fs and frozen in acid-washed polyethylene bottles until analysis. Samples for total phosphorus (TP) were preserved with 1µL of 5N H_2SO_4 per ml of sample, and refrigerated in acid-washed polyethylene bottles until analysis. Samples for dissolved organic carbon (DOC) were filtered through pre-combusted GF/Fs into pre-combusted glass vials and frozen until analysis. Whole water samples for dissolved inorganic carbon (DIC) analysis were preserved with mercuric chloride (25 ppm final concentration) in pre-combusted glass vials and refrigerated until analysis.

An index of the relative of amount of light and nutrients was calculated by dividing the proportion of open canopy at a site by either the soluble reactive phosphorus (SRP) or the dissolved inorganic nitrogen (DIN) concentration. This index allowed me to compare the light:nutrient environment among sites. Although many other variables such as water depth and turbidity, stream orientation, and latitude may contribute to the light environment at the stream benthos, percent canopy cover in shallow streams of similar latitudes accounts for most of the variation in light available for primary production. While total phosphorus is frequently used to indicate the amount of phosphorus that is available to algae (e.g., Sterner et al. 1997), I chose to use SRP in order to avoid problems with correlations among my variables. Total phosphorus includes suspended particulate phosphorus, which was also one of the dependent variables in my analyses.

Relationships between the C:N:P ratios of the stream algae and the various predictor variables were tested using backwards stepwise multiple regression analysis. One model was constructed for each C:nutrient ratio (periphyton and suspended matter C:P, C:N, and N:P). The suite of predictor variables entered was: the proportion of open canopy ("light"), the proportion of open canopy:SRP ("light:SRP"), the proportion of open canopy:DIN ("light:DIN"), DIC, DIN, SRP, DIC:DIN, DIC:SRP, and temperature. Each of these variables has the potential to influence either carbon fixation, nutrient assimilation, or the relative amounts of these two processes. Light availablility can influence photosynthetic rates and hence carbon fixation. Temperature also may influence photosynthesis, with higher temperatures leading to higher rates of carbon fixation. SRP and DIN are related to phosphorus and nitrogen availability, respectively. The predictor variables that are ratios have the potential to affect the relative amounts of

carbon fixation and nutrient assimilation. Variables were log transformed as necessary in order to meet the assumptions of normality and homogeneity of variances.

Nutrient limitation experiments

Nutrient limitation experiments were performed at ten of the 33 sites, using modified periphytometers (Matlock et al. 1998), as described in chapter 1. In the current experiment, I only manipulated nitrogen and phosphorus, but not light. The bottles were left in the streams for approximately two weeks, after which the filters were removed, transported back to the lab on ice, and then frozen for later analysis of chlorophyll *a*. The amount of chlorophyll per unit area was determined by dividing the chlorophyll *a* estimate by the surface area of the filters that was exposed through the bottle cap. To test for nutrient limitation, a factorial analysis of variance was performed on log-transformed chlorophyll data, with nitrogen and phosphorus as factors and rack as a blocking factor.

Results from the nutrient limitation experiments were compared to two different sets of guidelines proposed to predict nutrient limitation based on periphyton C:N:P, as outlined in Kahlert (1998) and Hillebrand and Sommer (1999). Kahlert based her guidelines on a literature review of freshwater benthic periphyton nutrient content (both stream and lake benthos). She included studies that indicated a change in periphyton C:N:P after nutrient enrichment, and also studies that used other nutrient limitation bioassays in addition to algal C:N:P data. A stream was predicted to be phosphorus limited if the C:P was greater than 369 or if the N:P was greater than 32. A nitrogen limited stream would have C:N greater than 11, or N:P less than 12. Both phosphorus and nitrogen limitation would be indicated by all ratios being relatively high (Kahlert

1998). The ratios determined by Hillebrand and Sommer (1999) to indicate nutrient limitation were similar to those of Kahlert for nitrogen limitation but were of a higher phosphorus content for phosphorus limitation. Using laboratory periphyton cultured from a Baltic Sea inoculum, they compared growth rates with C:N:P in order to determine optimum C:N:P ranges. If the periphyton N:P was less than 13 and the C:N was greater than 10, the periphyton were considered N limited. With an N:P greater than 22 and a C:P greater than 180, the periphyton were considered P limited. They suggest that both C:P and N:P must be high in order to predict P limitation, and likewise both C:N must be high and N:P low for N limitation. However, Kahlert's guidelines predict nutrient limitation if only one of the ratios is high (or low).

Chemical analyses

Samples for chlorophyll *a* were extracted in 95% acetone for 24 hours in the dark at 4°C, and measured fluorometrically (Turner Fluorometer Model 10-AU; Welschmeyer 1994). Soluble reactive phosphorus (SRP) was determined with the ascorbic acid method (American Public Health Association 1995) using an autoanalyzer (AlpKem Flow-3000). Total phosphorus (TP) and total dissolved phosporus (TDP) were put through a persulfate digestion (Wetzel and Likens 1979), then analyzed for SRP. Concentrations of nitrate plus nitrite nitrogen (NO_3^-/NO_2^--N) were analyzed by cadmium reduction followed by automated colorimetric analysis with the autoanalyzer, ammonium nitrogen (NH_4^+-N) was analyzed using the fluorometric method of Holmes et al. (1999), and dissolved reactive silica was measured by the molybdosilicate method (American Public Health Association 1995). Particulate carbon and nitrogen content was analyzed using a Perkin

Elmer 2400 CHN Elemental Analyzer. Dissolved inorganic carbon was analyzed on a Shimadzu total organic carbon analyzer (TOC-5000A), and dissolved organic carbon was analyzed as non-purgeable organic carbon on the same instrument.

Comparisons to other data sets

Periphyton and suspended matter C:N:P from these Minnesota streams were compared to lake seston and marine C:N:P discussed in Elser and Hassett (Elser and Hassett 1994) and Elser et al. (2000), using analysis of variance (ANOVA) and the Scheffé post-hoc comparisons test. For the analyses, I obtained the original data from the authors.

Results

Field survey

Canopy cover, discharge, water temperature, dissolved oxygen and pH values are presented in Table 2. All nutrients except for dissolved reactive silica varied significantly among regions (Table 3). Soluble reactive phosphorus ranged from 0.04 to 9.42 μM. Nitrate + nitrite-nitrogen ranged from 0.30 to 367 μM, and ammonium-nitrogen ranged from 0.70 to 15.9 μM. The dissolved phosphorus and nitrogen values were relatively low in the NE and NC streams, and quite high in the south. The two metro sites were intermediate.

Periphyton C:P site means ranged from approximately 100 to 450 (Figure 2). Although a few sites have larger standard errors due to some rocks that were high outliers, the mean C:P of most sites has a low standard error and ranges from about 100

to 300. Periphyton C:N site means ranged from 8 to 18, with most between 8 and 11. Similarly, a few sites displayed high variability, but the standard error of most sites was low compared to the mean. Periphyton N:P ranged from 15 to 27.

Differences existed between periphyton and suspended matter C:N:P. Suspended matter C:P was lower than periphyton C:P (paired t-test, $P < 0.0001$), with values ranging from approximately 50 to 275 (Figure 3). The range of suspended matter C:N was identical to that of periphyton C:N, 9 to 18, but suspended matter C:N was on average higher than periphyton C:N (paired t-test, $P < 0.0001$). Suspended matter N:P was lower than periphyton N:P (paired t-test, $P < 0.0001$). Suspended matter carbon:chlorophyll ratio site means varied more widely than the periphyton carbon:chlorophyll site means. (Figure 4).

The initial backwards stepwise multiple regression analysis yielded several significant predictor variables for periphyton and suspended matter C:N:P (Table 4). However, some of the significant relationships between the predictor and response variables were opposite than what would be expected according to mechanistic principles. For example, DIC was a significant predictor of suspended matter C:P, but higher levels of DIC led to lower suspended matter C:P. If carbon were limiting to the algae, I would expect higher DIC to increase the algal C:P, whereas if carbon were not limiting, additional carbon should not have an effect on algal C:P. My results do not support either of these scenarios. This unexpected relationship can be explained by regional differences. DIC and suspended matter C:P varied by region, with the NE streams having lower DIC, due to their location on the Canadian Shield, than both the NC and the southern streams (Table 3; Scheffé test, $P < 0.0001$ for both comparisons) and

higher average suspended matter C:P than the other regions (Figure 4; Scheffé test, all *P*-values with regard to the NE sites < 0.05).

I therefore removed from the regression models significant predictor variables based on their signs – ones that had a relationship with the response variable inconsistent with known mechanisms, and I ran the regressions again. Several of the independent variables were significant predictors of the periphyton and suspended matter C:N:P (Table 5). Some of the significant relationships, although not contrary to stoichiometric theory, are not relevant to the hypotheses being tested here regarding available light and nutrients. For example, SRP was a significant predictor of periphyton C:N. Therefore, I further examined only those relationships that had bearing on stoichiometric theory (indicated by an * after the *P*-value in Table 5).

Light was a significant predictor variable of periphyton C:N. As light levels increased, average periphyton C:N increased as well. However, the standard deviation of the periphyton C:N means was high relative to the means (Figure 6A), and the positive relationship suggested by the regression becomes meaningless when the data are viewed with error bars. Log SRP was a significant predictor of periphyton N:P, but, similar to the relationship in Figure 5A, the high standard deviations obscure the relationship (Figure 6B). On the other hand, the relationship between log SRP and suspended matter C:P remains evident even when plotted with standard deviations (Figure 6C). As SRP levels increased, suspended matter C:P decreased until approximately 50 to 150, after which further increases in SRP did not yield any changes in C:P . The periphyton samples were not as homogenous as the suspended matter samples, and this is reflected in the high variability of the periphyton nutrient ratios compared to that of the suspended matter.

Open vs. closed canopy sites

At the seven streams where I sampled both an open and a closed canopy reach, I predicted that periphyton C:nutrient ratios would be higher in the open canopy reach than in the closed canopy reach. Higher light availability, yet the same amount of dissolved nutrients, would lead to a higher available light:nutrient ratio, and, according to the light:nutrient hypothesis, would lead to higher algal C:nutrient. In one of the pairs of open and closed canopy sites, periphyton C:P was higher in the closed canopy site than in the open canopy site, contrary to the expectation that C:P would be higher in the higher light site (Fig.7A). There were no other significant differences in C:P between any of the other pairs of sites. Periphyton C:N was greater in the open canopy site than in the closed canopy site in two of the paired reaches (Fig. 7B). There were no significant differences between the remaining five pairs of sites.

Nutrient limitation experiments

Of the ten sites where I performed the nutrient limitation experiments, two were phosphorus limited, two were nitrogen limited, and the other six were neither nitrogen nor phosphorus limited (Table 6). At Chaska Creek there was a significant negative phosphorus effect. This stream had high TP (8.5 μM), and perhaps the additional P had a toxic effect on the periphyton. At three of the nutrient limited sites, Bear Creek (closed), Sucker Brook and West Branch of the Knife River, the increase in chlorophyll on the filters from the treatments with the limiting nutrient added was modest (70 to 140%).

However, in LS, there was a 700% increase in chlorophyll in the P-amended treatments (Table 6).

Using the nutrient limitation status guidelines from Kahlert (1998), periphyton C:N:P was able to correctly predict the nutrient limitation status in five of the nine sites where experiments were performed and from where I had periphyton C:N:P data (Table 7). In determining the number of sites where limitation was "correctly predicted," I included both the sites that were determined to be nutrient limited and the sites that were determined *not* to be nutrient limited by both my experiment and by the guidelines in the papers. According to the Hillebrand and Sommer (1999) guidelines, nutrient limitation was correctly predicted in five of the nine streams, although it is a different set of five streams than the set correctly predicted from Kahlert.

Comparisons to other aquatic environments

Stream periphyton and suspended matter C:N:P data averaged over all sites from this study were compared to lake and marine seston means, also averaged over all sites, discussed in Elser and Hassett (1994) and Elser et al. (2000). Means were compared using ANOVA and the Scheffé post-hoc comparisons test (Figure 8). Stream periphyton C:P, C:N and N:P did not differ from lake seston. Stream periphyton and suspended matter differed from one another with regard to all three ratios, and the magnitude of those differences was as great as the magnitude of differences between lake and marine seston. The magnitude of difference among the C:P and N:P means was greater than that of C:N.

Discussion

Stream algae C:N:P

In this set of Minnesota streams, I found that the periphyton C:N:P differs in a predictable manner from the suspended matter C:N:P, and that patterns of nutrient limitation can influence the algal elemental composition.

There were several significant predictors of periphyton and suspended matter C:N:P (Table 5), but when the variability within the individual data points in the regressions was examined, the relationships between light and periphyton C:N, and SRP and periphyton N:P were lost (Figure 6A, B). However, the relationship between SRP and suspended matter C:P holds (Figure 6C). Higher levels of SRP were associated with lower C:P until approximately a C:P of 50 to 150, after which further increases in SRP did not lead to lower suspended matter C:P. Two possible explanations of this relationship are discussed in the next section. Due to the "NE" signature of lower DIC levels and lower SRP, this pattern was initially obscured by the stronger relationship between suspended matter C:P and DIC (Figure 5).

I was interested in whether or not the light:nutrient hypothesis presented by Sterner and colleagues (1997) would hold in these streams. I focus on periphyton here; since suspended matter flows downstream, the light environment that I characterized at the sampling site is not necessarily the light environment recently experienced by the suspended matter. One would expect the relationships predicted by the light:nutrient hypothesis to hold only if the system is both nutrient and light limited. Under nutrient and light limitation, carbon fixation and nutrient assimilation are uncoupled and higher growth rates (due to increased light intensity) or lower available nutrients yield higher

algal C:nutrient. If the system is not nutrient limited, then as algal growth rates increase due to higher light intensity, the algae are able to assimilate more nutrient, holding the C:nutrient ratio relatively stable. If the system is not light limited, then an increase in light will not increase the algal growth capacity or growth rate and therefore will not affect biomass C:nutrient. In my study there was not a clear response of periphyton C:N:P to light or to the balance of light and nutrients (Table 5). Periphyton C:N responded positively to light in only two of the seven pairs of sites (Figure 7). In one open canopy site, periphyton C:P was actually *lower* than in the closed canopy site, contrary to expectations. Hill and Knight (1988) found a trend of higher periphyton biomass in artificially shaded sites, and they suggest photoinhibition of the algae in the high light sites as a possible explanation. Perhaps the higher light intensities in the open canopy sites decreased photosynthetic rates and lowered periphyton C:P.

The lack of a relationship in my study between light:nutrient and periphyton C:N:P does not agree with results that Sterner et al. (1997) found for lake seston in which there was a significant correlation between light:nutrient (in their case, the ratio of mixed-layer mean light to total phosphorus) and seston C:P. Several factors could be driving these results. Canopy cover might not accurately describe the periphyton light environment at my sites; light extinction in the water column might have had more of an influence on total available light than I had expected. It is also possible that the periphyton were not light limited, even in the closed canopy sites. Sunflecks due to light passing through the canopy can account for a substantial amount of periphyton photosynthesis (Wellnitz and Rinne 1999). Periphyton in shallow streams with an open canopy may be less likely to be light-limited than lake phytoplankton, which are

distributed throughout the mixed layer, and often occur at depths up to tens of meters. Therefore, although there is often no "canopy cover" shading a lake, the light available for photosynthesis depends on the depth at which the phytoplankton are located. Conversely, periphyton in shallow streams remain close to the surface of the water where the light intensity has attenuated less.

Additionally, as previously discussed, I would not expect light:nutrient to influence algal C:nutrient under an absence of nutrient limitation. However, four of the ten sites where nutrient limitation experiments were performed were nutrient limited, and despite this nutrient limitation, the periphyton C:limiting nutrient at two of these streams was not greater in the open canopy site than in the closed canopy site (BEAR and WBK, Figure 7). Open and closed canopy sites were not sampled for the other two nutrient limited sites, but the periphyton C:N:P of these sites can be compared to other sites in the data set. In Sucker Brook (SCK), the C:limiting nutrient (nitrogen) was 9.5. The periphyton C:N at the non-nitrogen limited experimental sites ranged from 8.8 to 16.7, and the C:N of Sucker Brook of 9.5 falls at the lower end of this range. If nutrient limitation had the predicted effect on periphyton C:N at Sucker Brook, I would have expected the C:N to be higher. For the fourth nutrient (phosphorus) limited site, La Salle (LS), I do not have periphyton data from the study. However, it was sampled the previous year during a pilot study (unpublished). The periphyton C:P average was 540, significantly higher than three of the four other sites (Scheffé post-hoc comparisons, $P <$ 0.00001 for three of the four comparisons with LS) that I sampled that year, and also significantly higher than all but two sites from the larger data set of the current study

(Scheffé post-hoc comparisons, $P < 0.00001$ for all but two of the 31 comparisons with LS). Why did I see higher C:nutrient here but not in the other nutrient limited sites?

In the nutrient limitation experiments, there was a 700% increase in chlorophyll *a* in the P-amended treatments at La Salle, whereas in the other three nutrient limited sites the addition of the limiting nutrient led to chlorophyll increases of only 70 to 140% (Table 6). Goldman et al. (1979) demonstrated that more severely nutrient limited algae will have a higher C:nutrient than algae exposed to a lower degree of nutrient limitation. My results agree with their findings in that the degree of nutrient limitation at La Salle was much higher than at the other three sites, as measured by the relative increase in primary production when the limiting nutrient was supplied in excess. SRP concentrations in La Salle were 0.04 μM, which is the method detection limit, and this low concentration could account for the severity of the P limitation. The low SRP levels are probably due to the stream's location. La Salle drains La Salle Lake approximately 100 meters upstream from the sampling location and this water is most likely epilimnetic water low in dissolved nutrients from the summer stratification period. Perhaps the nutrient limitation in some of my streams, even though it does exist, is not strong enough to allow me to detect a higher periphyton C:nutrient.

Other factors that I did not measure could influence the periphyton and suspended matter elemental composition. In streamside flow-through channels, Rosemond (1993) found that grazing by snails decreased the periphyton C:N. In an experiment with laboratory stream channels (Mulholland et al. 1991), periphyton %N and %P were higher in treatments with snails compared to those without the snails. The authors speculate that this was due to the removal of senescent algae by grazers. Steinman et al. (1987) found

an opposite effect of grazers in that periphyton C:N was highest in one of the high density grazer treatments. Since I did not investige the effect of grazing in my study, I can not conclude that it did not influence algal C:N:P in my sites.

My sampling regime was another potential source of error. I sampled each stream only one time, and I sampled most of the sites in a region within a two week period. Therefore, some of the regional differences might be attributed to seasonal variation as opposed to specific characteristics of the region. However, I am not as interested in regional differences as I am in how current conditions influence stream stoichiometry across a wide range of physcial and chemical conditions.

Differences between periphyton and suspended matter C:N:P

The elemental composition of the stream suspended matter and periphyton differed in that periphyton C:P was higher than suspended matter C:P, yet periphyton C:N was lower than that of the suspended matter (Figure 8A, B). This pattern suggests that the underlying difference between the two groups is in terms of N and P; the suspended matter N:P was lower than the periphyton N:P. Two different explanations could account for this pattern, one abiotic and the other biotic.

The suspended matter may carry a proportionally larger load of inorganic phosphorus than the periphyton, in the form of either suspended particulates that contain inorganic phosphorus, or as phosphates adsorbed to suspended biotic particles. Phosphates tend to adsorb to particles, whereas inorganic nitrogen does not. The suspended particulate matter might be smaller in size than the particulate matter in the periphyton matrix, hence a greater surface area to which the phosphates could adsorb.

Additionally, turbulence could lead to a greater amount of particulates in suspension. These scenarios could cause the different N:P ratios, would account for the rather low suspended matter C:P, and would also explain the relationship between SRP and suspended matter C:P (Figure 6C). At higher concentrations of SRP, there is more inorganic phosphate available to sorb to particles, hence the lower suspended C:P.

Alternatively, the underlying cause of these patterns could be biological. It is generally believed that suspended algae in streams are cells originating from a benthic periphyton population (Swanson and Bachmann 1976). If these cells are not actively growing or respiring, I would expect their C:N:P to be similar to that of the periphyton. Alternatively, if they are senescent cells sloughed-off from the benthos, I might expect them to have lower nutrient concentrations than the periphyton (Hunter 1980). However, I found neither of these to be the case. The suspended matter was higher in C:N but lower in C:P (lower N:P) than the periphyton. It appears that after it is sloughed-off from the periphyton, the suspended matter takes up more phosphorus relative to nitrogen.

If this is the case, it suggests that the suspended matter is living and actively growing biota. The higher phosphorus concentrations in the suspended matter could be due to it being exposed to a higher concentration of available phosphorus than the periphyton. These two communities are exposed to the same bulk water, yet the nutrient concentrations in their immediate environment may be different due to boundary layers. As defined in Vogel (1981), the thickness of a boundary layer increases in proportion to the square root of the distance from the leading edge, and decreases with an increase in velocity. In the case of epilithic periphyton, the "distance from the leading edge" refers to the length of the rock in the direction that is parallel to flow. Therefore, the boundary

layer of cells in an epilithic periphyton matrix would be thicker than the boundary layer of a cell suspended in water, since the "distance from the leading edge" in that case would be the length of the cell, or the length of an aggregation of cells. Applying Fick's Law of Diffusion (Cutnell and Johnson 1995) to boundary layers, the amount of nutrient that diffuses through a boundary layer and reaches a cell's surface is inversely proportional to the thickness of the boundary layer. If nutrient uptake within the rock's boundary layer is greater than the rate at which nutrient is resupplied from the water column, then the nutrient concentration will be lower in the rock's boundary layer than in the water column. Hecky and Hesslein (1995) have shown that thicker boundary layers may slow inorganic carbon transport to cells and may increase carbon limitation. Likewise, if, within the boundary layer, the periphyton are phosphorus limited, yet have sufficient inorganic carbon and nitrogen, higher C:P and higher N:P would be expected in the periphyton.

I would therefore expect C:P ratios to be even higher in phosphorus limited lake periphyton. Periphyton in a lake benthos exists in the boundary layer of its substrate, yet it does not have the benefit of flowing water that stream periphyton have. An increase in velocity will decrease the thickness of a boundary layer, and it has been demonstrated that flowing water can increase the nutrient supply to periphyton (Stevenson and Glover 1993), can increase the nutrient uptake of phosphorus-deficient periphyton (Borchardt et al. 1994), and can decrease macrophyte C:N (Parker 1981). In one study of lake periphyton in an oligotrophic Canadian Shield lake, mean periphyton C:P grown on artificial substrata was approximately 450 (Frost and Elser in press). This value is at the

upper end of the periphyton C:P range in my sites (Figure 2A), and is higher than all but one of the periphyton site means in my study.

Other factors may differentially affect periphyton and suspended matter nutrient content. Periphyton density can influence transport rates of nutrients through a periphyton mat (Stevenson and Glover 1993) and could therefore influence nutrient availability to the algae. Additionally, some periphyton species might produce carbon-rich mucilaginous polysaccharides (Bothwell 1985) which would increase the overall periphyton C:nutrient content. The relative amount of bacteria in the periphyton compared to the suspended matter would also affect the elemental composition in that bacteria generally have lower C:nutrient content than algae (Chrzanowski and Kyle 1996). Nitrogen content could be influenced by the existence of nitrogen-fixers in the periphyton.

Comparisons to other aquatic environments

The stream suspended matter in my data set had more phosphorus and less nitrogen than the lake seston described in Elser et al. (2000) (Figure 8). Stronger phosphorus limitation in lakes vs. streams could account for the differences in phosphorus content, and is supported by the observation that a majority of my sites were not phosphorus limited; only two of the ten sites in which I performed the nutrient limitation experiments were phosphorus limited. It is unclear what is driving the lower nitrogen and higher phosphorus content in stream suspended matter vs. both lake and marine seston.

Stream periphyton C:N:P means were not statistically different from the lake seston means (Figure 8). This may also be related to nutrient limitation. As discussed in the previous section, although stream suspended matter may not be nutrient limited, stream periphyton exposed to the same bulk water as the suspended matter may be nutrient limited, due to boundary layers. Perhaps the stream periphyton and lake seston C:N:P ratios are similar due to both of these communities existing under nutrient limited conditions.

Use of algal C:N:P to predict nutrient limitation

The guidelines from both Kahlert (1998) and Hillebrand and Sommer (1999) correctly predicted nutrient limitation status of the periphyton in five of the nine streams (Table 7). The Kahlert guidelines are based on a literature review of studies in which periphyton C:N:P and nutrient limitation in both streams and lakes were assessed, and the Hillebrand and Sommer guidelines are based on laboratory experiments with semicontinuous dilution cultures of benthic microalgae. The algae in their experiments were therefore more analogous to lake periphyton than stream periphyton. This, in addition to the inclusion of both lake and stream periphyton in the Kahlert guidelines, might account for their predictions not consistently agreeing with the results of my study. As previously discussed, flow can influence nutrient supply to benthic algae (Stevenson and Glover 1993), and possibly alter their elemental composition. Both the Kahlert (1998) and the Hillebrand and Sommer (1999) papers state that their guidelines are preliminary. I suggest that a separate set of guidelines should be developed for both

streams and lake periphyton, due to the possibility that the different environments lead to different algal nutrient composition.

Similarly, Francoeur et al. (1999) found periphyton N:P in natural communities to be a poor predictor of nutrient limitation. Nitrogen and phosphorus limitation and co-limitation, as assessed with nutrient diffusing substrata, occurred over a wide range of periphyton N:P ratios. They suggest that a potential large source of error is the inclusion of non-algal nitrogen and phosphorus in the periphyton samples. The presence of detritus, microbes, and extracellular polysaccharides may alter periphyton C:N:P ratios. Therefore, other complementary methods, such as nutrient diffusing substrata, should be used before concluding nutrient limitation based on periphyton C:N:P ratios alone.

The experiments that I performed to determine nutrient limitation illustrate the short-term response of the periphyton community to a relatively large addition of nutrients. However, nutrient limitation can be measured in other ways. For example, Chapin et al. (1986) point out that the degree of nutrient limitation of a natural plant community depends on its species composition. As the amount of available nutrient increases, species replacement may change the community composition, and therefore the maximum growth potential of the community may change as well. One must keep in mind these different ideas of nutrient limitation when interpreting results of nutrient limitation experiments.

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Table 1. List of regions, site names and abbreviations, and latitude and longitude.

Region	*Abbreviation*	*Site*	*Latitude*	*Longitude*
NE	AM	Amity Creek	46.86	-92.02
	CLOQ	Feeder stream to Otter Creek at Cloquet Forestry Center	46.69	-92.49
	DS	Dutch Slough	46.80	-92.45
	ELM	Elm Creek	46.76	-92.33
	KNAT	Keenes Creek, Keenes Creek Park	46.77	-92.18
	KREC	Keenes Creek, Irving Community Center	46.73	-92.17
	KRLY	Keenes Creek, upstream	46.79	-92.18
	TCNC CL	Tischer's Creek, Hartley Nature Center, closed canopy	46.84	-92.08
	TCNC OP	Tischer's Creek, Hartley Nature Center, open canopy	46.84	-92.08
	TCP	Tischer's Creek, Hartley Park	46.83	-92.09
	WBK CL	West Branch Knife River, closed canopy	47.05	-91.86
	WBK OP	West Branch Knife River, open canopy	47.05	-91.86
	WPR	White Pine River	46.80	-92.45
NC	BEAR CL	Bear Creek, closed canopy	47.35	-95.23
	BEAR OP	Bear Creek, open canopy	47.35	-95.23
	CPL	Mississippi River, at Coffee Pot Landing	47.35	-95.18
	HIER	Hier Creek	47.34	-95.33
	HLC	Heart Lake Creek	47.29	-95.32
	LS	La Salle Creek	47.35	-95.17
	LSP CL	La Salle Creek, Itasca State Park, closed canopy	47.24	-95.16
	LSP OP	La Salle Creek, Itasca State Park, open canopy	47.24	-95.16
	MISS	Mississippi, at Rt. 2 crossing	47.33	-95.22
	MLC CL	Mud Lake Creek, closed canopy	47.29	-95.32
	MLC OP	Mud Lake Creek, open canopy	47.29	-95.32
	NIC	Nicollet Creek	47.19	-95.23
	SCK	Sucker Brook	47.25	-95.26
Metro	BAS	Bassett Creek	45.00	-93.33
	MINN	Minnehaha Creek, east of I-35	44.91	-93.27
South	CARV CL	Carver Creek, closed canopy	44.78	-93.73
	CARV OP	Carver Creek, open canopy	44.78	-93.73
	CARV UP	Carver Creek, upstream	44.80	-93.75
	CHAS CL	Chaska Creek, closed canopy	44.79	-93.61
	CHAS OP	Chaska Creek, open canopy	44.79	-93.61
	SILV	Silver Creek	44.69	-93.74

Table 2. Canopy cover, discharge, water temperature, percent dissolved oxygen, and pH of all streams sampled. Missing pH values are due to malfunctioning of the meter. Site abbreviations as in Table 1.

Region	Abbreviation	Proportion open canopy	Discharge (m^3/s)	Water temperature (°C)	Percent dissolved oxygen	pH
NE	AM	0.59	0.111	8.5	97	8.0
	CLOQ	0.18	0.022	6.8	66	7.2
	DS	0.98	0.559	14.1	88	7.3
	ELM	0.83	0.376	12.7	85	8.3
	KNAT	0.49	0.033	12.3	85	8.1
	KREC	0.79	0.186	10	97	8.4
	KRLY	0.40	0.010	11.1	75	7.8
	TCNC CL	0.42	0.048	12.9	92	7.6
	TCNC OP	0.90	0.039	12.1	91	7.6
	TCP	0.34	0.009	12.7	87	8.0
	WBK CL	0.34	0.210	13.6	79	7.6
	WBK OP	0.84	0.162	13.3	80	7.6
	WPR	0.69	0.593	13.8	96	8.1
NC	BEAR CL	0.73	0.818	17	68	8.3
	BEAR OP	0.99	0.042	17.3	68	8.2
	CPL	0.98	1.321	20.6	78	8.3
	HIER	1.00	0.194	20.9	42	7.9
	HLC	0.07	0.100	19.9	56	7.5
	LS	0.92	0.368	24.7	93	8.4
	LSP CL	0.22	0.076	20.1	76	7.8
	LSP OP	0.91	0.088	17.8	73	7.9
	MISS	0.98	1.334	19.7	82	8.3
	MLC CL	0.49	0.090	18	36	8.0
	MLC OP	0.93	0.054	19.3	43	7.8
	NIC	0.98	0.087	20.4	79	7.7
	SCK	0.98	0.270	20.1	70	8.0
Metro	BAS	0.32	1.095	22.5	80	
	MINN	0.48	0.182	21.1	50	
South	CARV CL	0.45	0.187	19.9	71	8.1
	CARV OP	0.86	0.179	19.1	62	8.0
	CARV UP	0.52	0.058	17.1	76	
	CHAS CL	0.34	0.135	16.7	89	8.3
	CHAS OP	1.00	0.066	15.5	84	8.2
	SILV	0.54	0.057	18.7	112	

Table 3. Average nutrient concentrations at each site, N = 2 for all means. DIC = dissolved inorganic carbon, DOC = dissolved organic carbon, DRSi = dissolved reactive silica, NO_3^-/ NO_2^--N = nitrate + nitrite-nitrogen, NH_4^+-N = ammonium-nitrogen, SRP = soluble reactive phosphorus, TP = total phosphorus, DIN = nitrate + nitrite + ammonium-nitrogen. *P*-values are results of a one-way ANOVA for each nutrient, with region as the predictor variable. Site abbreviations as in Table 1. (Continued on next page.)

Region	*Site*	*DIC (mM)*	*DOC (mM)*	*DRSi (mM)*	*NO_3^-/ NO_2^--N (µM)*	*NH_4^+-N (µM)*	*SRP (µM)*	*TP (µM)*	*DIN/SRP*
	P-values:	< 0.0001	0.05	0.12	< 0.0001	0.003	< 0.0001	< 0.0001	< 0.0001
NE	AM	1.46	0.56	0.14	2.07	4.34	0.10	0.08	66.97
	CLOQ	0.52	0.82	0.12	4.17	1.42	0.31	0.29	17.75
	DS	1.43	0.84	0.10	1.56	1.80	0.37	0.55	9.17
	ELM	1.93	0.64	0.16	5.86	1.48	0.20	0.38	36.93
	KNAT	1.36	0.79	0.16	9.29	0.70	0.17	0.37	60.23
	KREC	1.57	0.49	0.18	10.05	1.06	0.10	0.19	112.64
	KRLY	1.01	0.93	0.11	1.28	1.15	0.15	0.18	16.59
	TCNC CL	1.45	0.88	0.11	3.07	1.40	0.15	0.50	30.72
	TCNC OP	1.45	0.88	0.10	3.11	1.28	0.11	0.48	40.27
	TCP	1.39	0.70	0.07	2.05	1.43	0.29	0.67	12.18
	WBK CL	0.82	1.42	0.10	2.72	0.96	0.05	0.26	78.48
	WBK OP	0.82	0.91	0.10	2.73	1.11	0.06	0.23	62.64
	WPR	1.38	0.84	0.08	1.52	0.80	0.11	0.29	21.21
NC	BEAR CL	5.96	1.15	0.31	1.28	1.66	1.08	1.36	2.72
	BEAR OP	5.95	1.19	0.34	1.68	1.53	1.09	1.51	2.96
	CPL	4.22	0.58	0.24	1.00	1.25	0.94	1.65	2.39
	HIER	3.49	0.82	0.17	0.50	1.90	0.83	1.09	2.90
	HLC	2.86	0.63	0.08	0.35	1.18	0.36	0.68	4.28
	LS	3.50	0.37	0.21	0.30	2.47	0.04	0.14	66.39
	LSP CL	5.12	0.36	0.21	0.82	1.64	0.33	0.56	7.39
	LSP OP	5.18	0.33	0.23	0.98	2.32	0.31	0.58	10.57
	MISS	3.95	0.46	0.18	0.77	1.64	0.62	1.86	3.87
	MLC CL	4.60	0.70	0.20	2.24	2.26	0.27	0.61	16.97
	MLC OP	4.83	0.63	0.23	2.08	2.41	0.23	0.57	19.31
	NIC	4.55	0.48	0.26	2.16	2.15	0.57	1.29	7.52
	SCK	3.51	0.48	0.14	0.92	2.32	0.23	0.73	14.28

Table 3. Continued from previous page.

Region	*Site*	*DIC (mM)*	*DOC (mM)*	*DRSi (mM)*	*NO_3^-/ NO_2^--N (μM)*	*NH_4^+-N (μM)*	*SRP (μM)*	*TP (μM)*	*DIN/SRP*
Metro	BAS	2.80	0.56	0.16	11.04	4.01	0.83	3.00	18.16
	MINN	2.42	0.54	0.05	9.73	2.46	0.36	1.06	34.25
South	CARV CL	4.85	1.43	0.02	111.42	15.88	9.32	12.73	13.66
	CARV OP	4.82	1.39	0.02	110.78	15.52	9.42	12.24	13.41
	CARV UP	5.60	1.29	0.14	48.30	2.42	9.16	12.96	5.54
	CHAS CL	6.18	0.69	0.39	112.12	2.75	6.50	9.69	17.68
	CHAS OP	6.44	0.66	0.39	110.63	3.60	5.47	8.54	20.88
	SILV	6.24	0.68	0.32	366.95	0.82	6.65	7.68	55.30

Table 4. *P*-values from backward stepwise multiple regressions on periphyton and suspended matter C:N:P. All predictor variables were entered in these six models, and the displayed *P*-values are for those variables that remained in the model. The *P*-values in bold indicate those predictors that were removed for the next set of regressions (see text).

	Periphyton			*Suspended matter*		
Predictor	*C:P*	*C:N*	*N:P*	*C:P*	*C:N*	*N:P*
Light	-	0.0009	-	-	-	0.0009
Light:SRP	-	-	-	-	-	0.0002
Light:DIN	-	-	-	-	-	-
DIC	-	-	-	**<0.0001**	0.0015	**<0.0001**
log DIN	-	-	-	-	-	-
log SRP	-	-	0.0006	-	-	-
DIC:DIN	-	-	-	-	-	-
DIC:SRP	-	**0.0029**				-
Temperature	-	-	-	-	**0.0031**	-

Table 5. *P*-values from backward stepwise multiple regressions on periphyton and suspended matter C:N:P, after removal of the predictors indicated in Table 4. *P*-values with a * indicate those predictors that are further explored in the text.

	Periphyton			*Suspended matter*		
Predictor	*C:P*	*C:N*	*N:P*	*C:P*	*C:N*	*N:P*
Light	-	0.002*	-	-	-	0.001
Light:SRP	-	-	-	-	-	< 0.001
Light:DIN	-	-	-	-	-	-
DIC	-	-	-	-	-	-
log DIN	-	-	-	-	-	-
log SRP	-	0.001	0.001*	0.003*	-	-
DIC:DIN	-	-	-	-	-	-
DIC:SRP	-	-	-	-	-	-
Temperature	-	-	-	-	-	-

Table 6. Average µg chlorophyll *a* ± SE on filters at the end of nutrient limitation experiments. % change is the direction of change and percent difference between treatments without the limiting nutrient and treatments with the limiting nutrient added. Cells marked with a * indicate a significant ANOVA main effect (either N or P) at that site. * $P < 0.01$, ** $P < 0.0001$.

Site	*Region*	*control*	*N*	*P*	*N + P*	*% change*
DS	NE	2.7 ± 0.29	3.3 ± 0.79	2.9 ± 0.46	4.1 ± 1.1	
KREC	NE	4.4 ± 1.1	5.3 ± 1.5	4.4 ± 0.56	6.2 ± 1.7	
KRLY	NE	0.92 ± 0.22	2.4 ± 0.73	1.7 ± 0.40	1.1 ± 0.17	
WBK CL	NE	2.2 ± 0.66	1.7 ± 0.20	3.9 ± 0.29*	2.8 ± 0.40	+70%
BEAR CL	NC	3.1 ± 0.51	8.9 ± 1.7**	2.5 ± 0.48	7.4 ± 0.74	+ 90%
LS	NC	1.6 ± 0.18	1.3 ± 0.13	9.0 ± 0.99**	14 ± 3.3	+700%
NIC	NC	1.5 ± 0.51	1.5 ± 0.72	1.7 ± 0.48	1.5 ± 0.42	
SCK	NC	3.7 ± 0.25	7.6 ± 1.2**	3.4 ± 0.34	9.4 ± 2.6	+140%
CARV OP	South	5.6 ± 1.4	5.9 ± 0.71	5.7 ± 1.4	6.2 ± 1.0	
CHAS OP	South	8.1 ± 3.4	3.9 ± 1.1	1.8 ± 0.21*	1.6 ± 0.45	- 70%

Table 7. Predicted and actual limiting nutrients for the 9 sites where the nutrient limitation experiments were performed and from where we have periphyton C:N:P data. Predicted limiting nutrients are based on periphyton C:N:P guidelines in Kahlert 1998 and Hillebrand and Sommer (1999). Actual limiting nutrient data from the nutrient limitation experiments in this study. LS periphyton C:N:P data from a 1999 pilot study (unpublished).

Site	Predicted limiting nutrient (Kahlert 1998)	Predicted limiting nutrient (Hillebrand and Sommer 1999)	Actual limiting nutrient (experimental data)
DS	-	-	-
KREC	-	P	-
WBK CL	P	P	P
CHAS OP	N	-	-
CARV OP	-	-	-
BEAR CL	-	-	N
LS	P	P	P
SCK	-	P	N
NIC	N+P	P	-

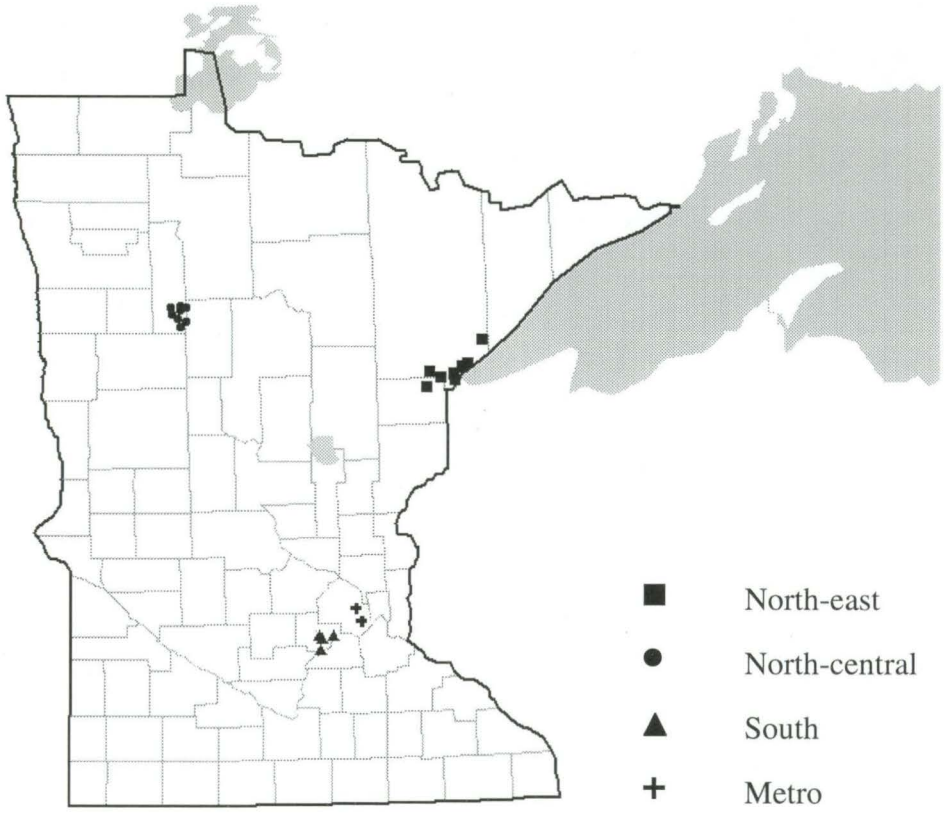

Figure 1. Location of sites by region

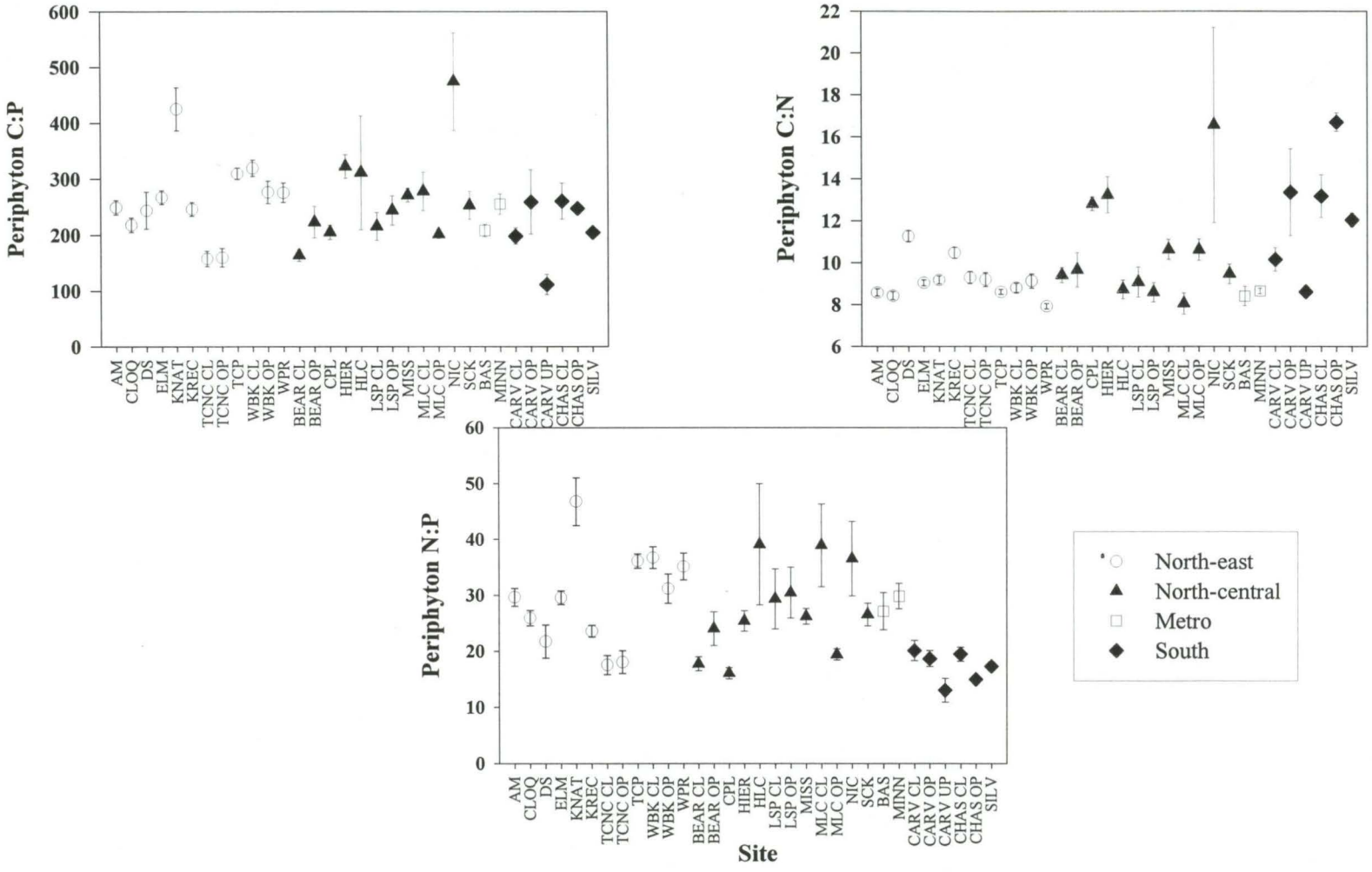

Figure 2. Periphyton (A) C:P, (B) C:N, and (C) N:P (means +/- SE) by site, and grouped according to geographic region (in order to highlight regional differences). Within a region, sites are listed alphabetically. N = 8 rocks at most sites.

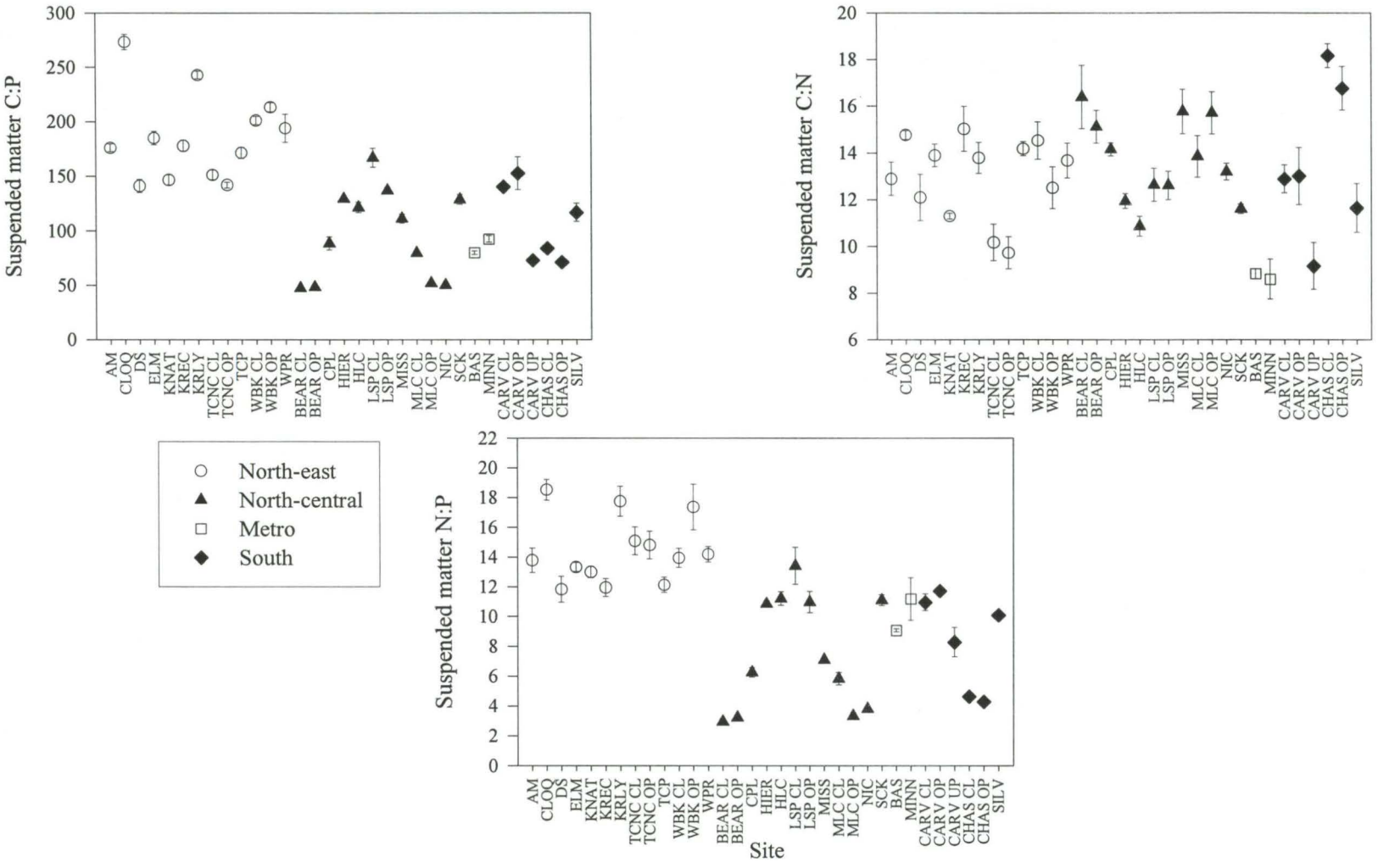

Figure 3. Suspended matter (A) C:P, (B) C:N, and (C) N:P (means +/- SE) by site, and grouped according to geographic region (in order to highlight regional differences). Within a region, sites are listed alphabetically. N = 2 at each site.

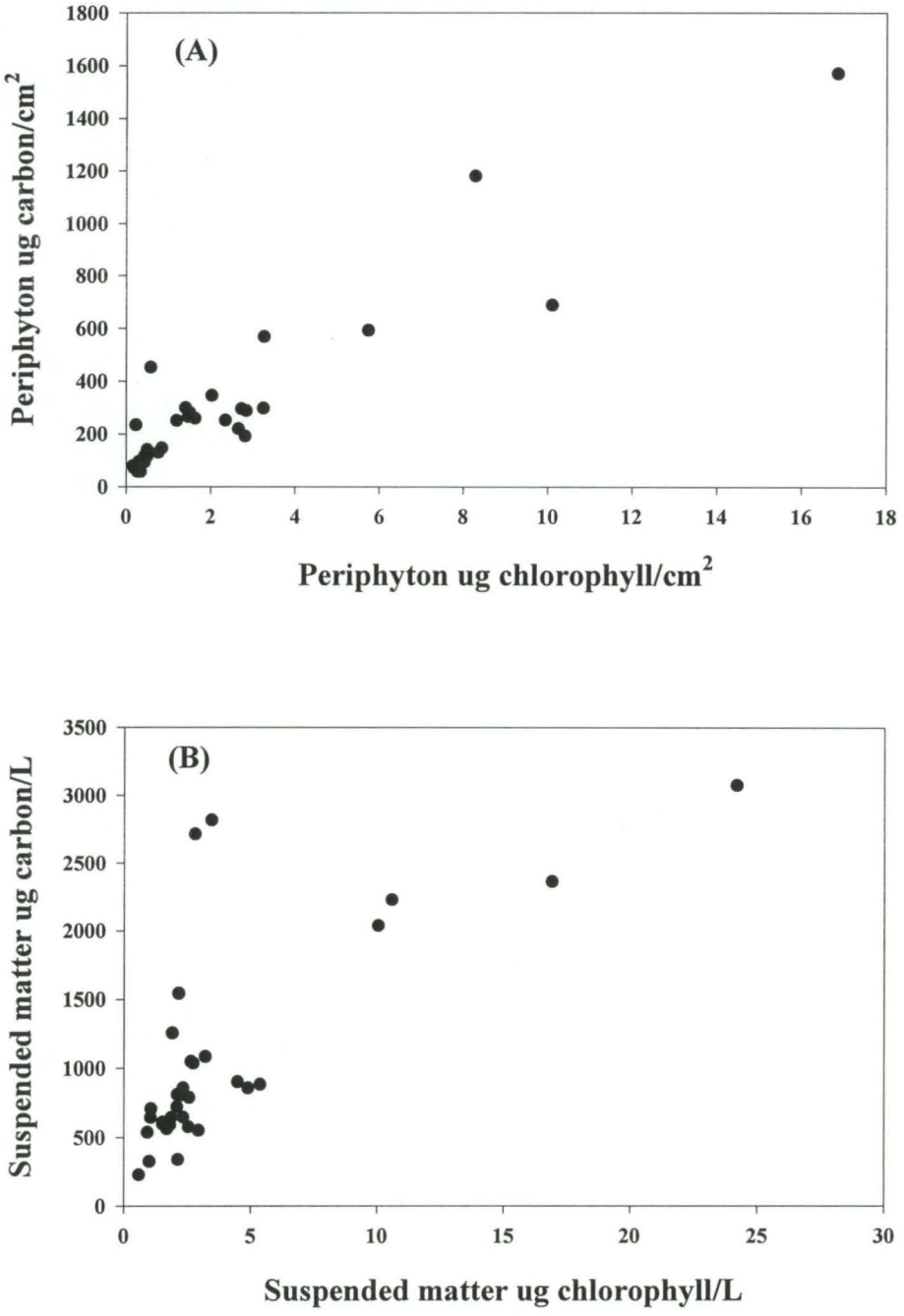

Figure 4. Periphyton (A) and suspended matter (B) carbon and chlorophyll content. Each point represents a site mean.

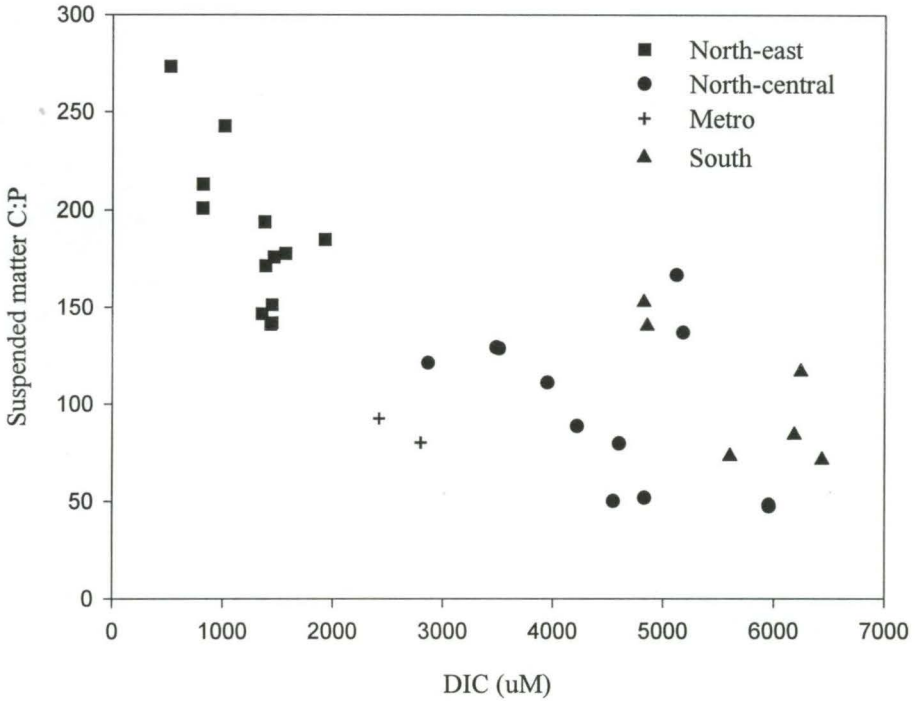

Figure 5. Site average DIC vs. suspended matter C:P, grouped by region.

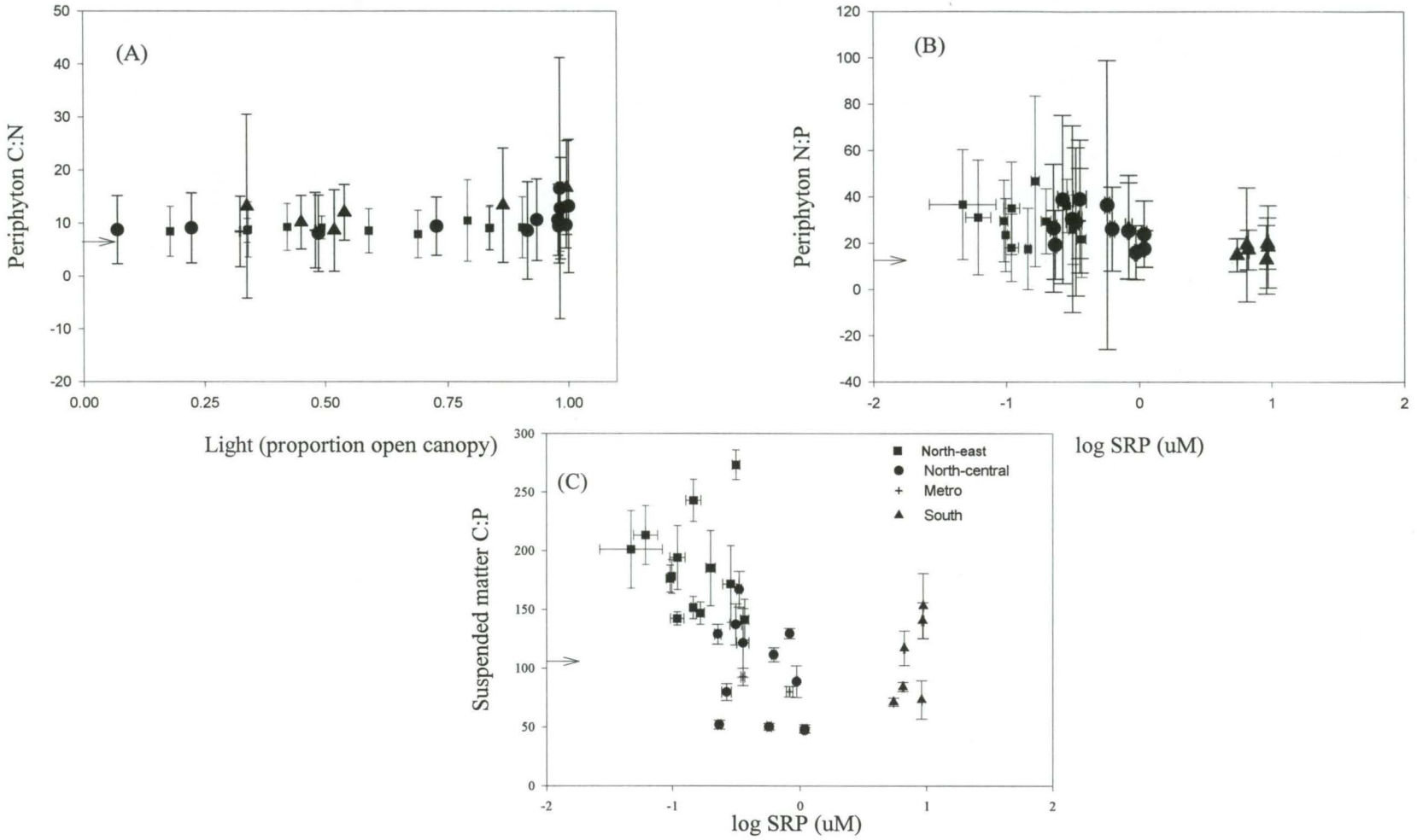

Figure 6. (A) Light vs. periphyton C:N, (B) log SRP vs. periphyton N:P, and (C) log SRP vs. suspended matter C:P. All values except for light are mean +/- SD. Ratio SDs calculated through error propagation (Bevington 1969). Arrows indicate C:N:P Redfield ratio of 106:16:1. Means grouped by region, legend in 5C.

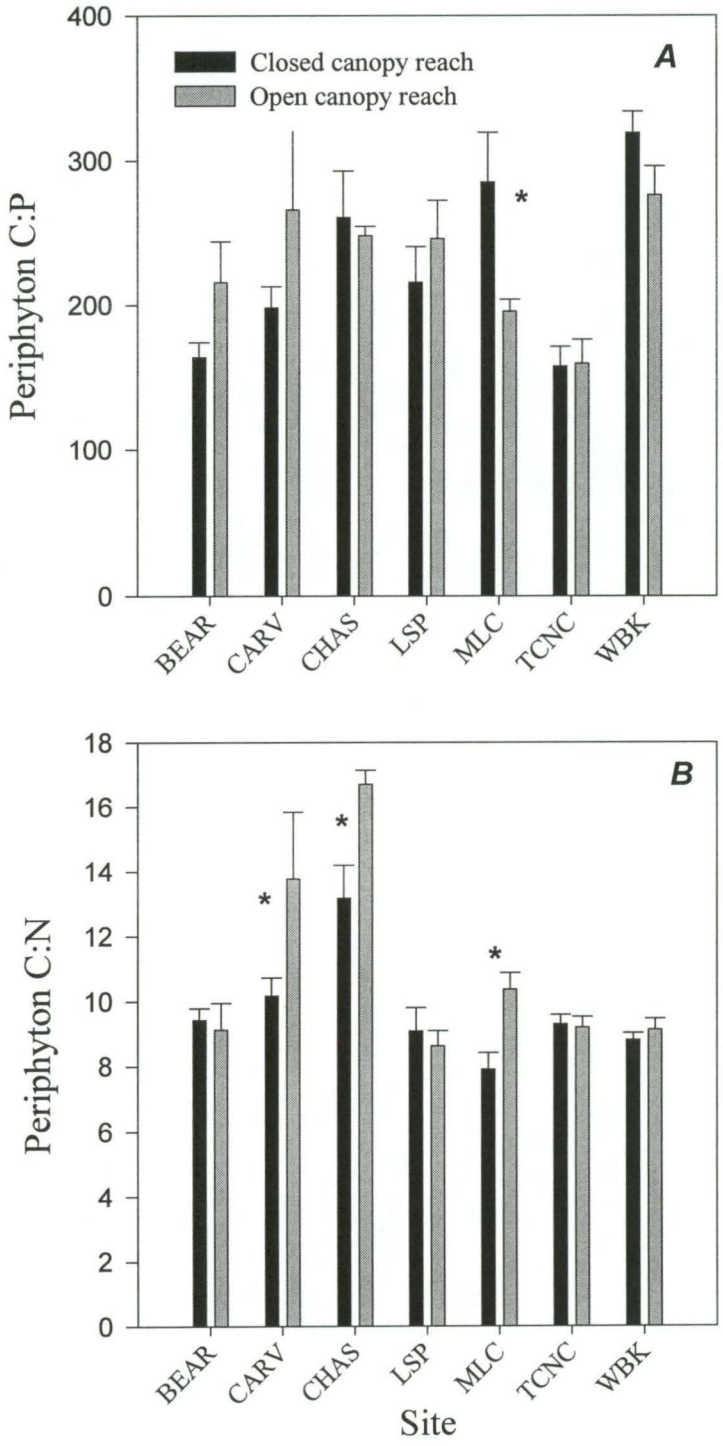

Figure 7. Periphyton (A) C:P and (B) C:N ratios in the 7 pairs of closed and open canopy sites. Bars represent means +/- SE. * indicates that the open and closed canopy reaches within a pair are significantly different from one another at $P < 0.05$, t-test.

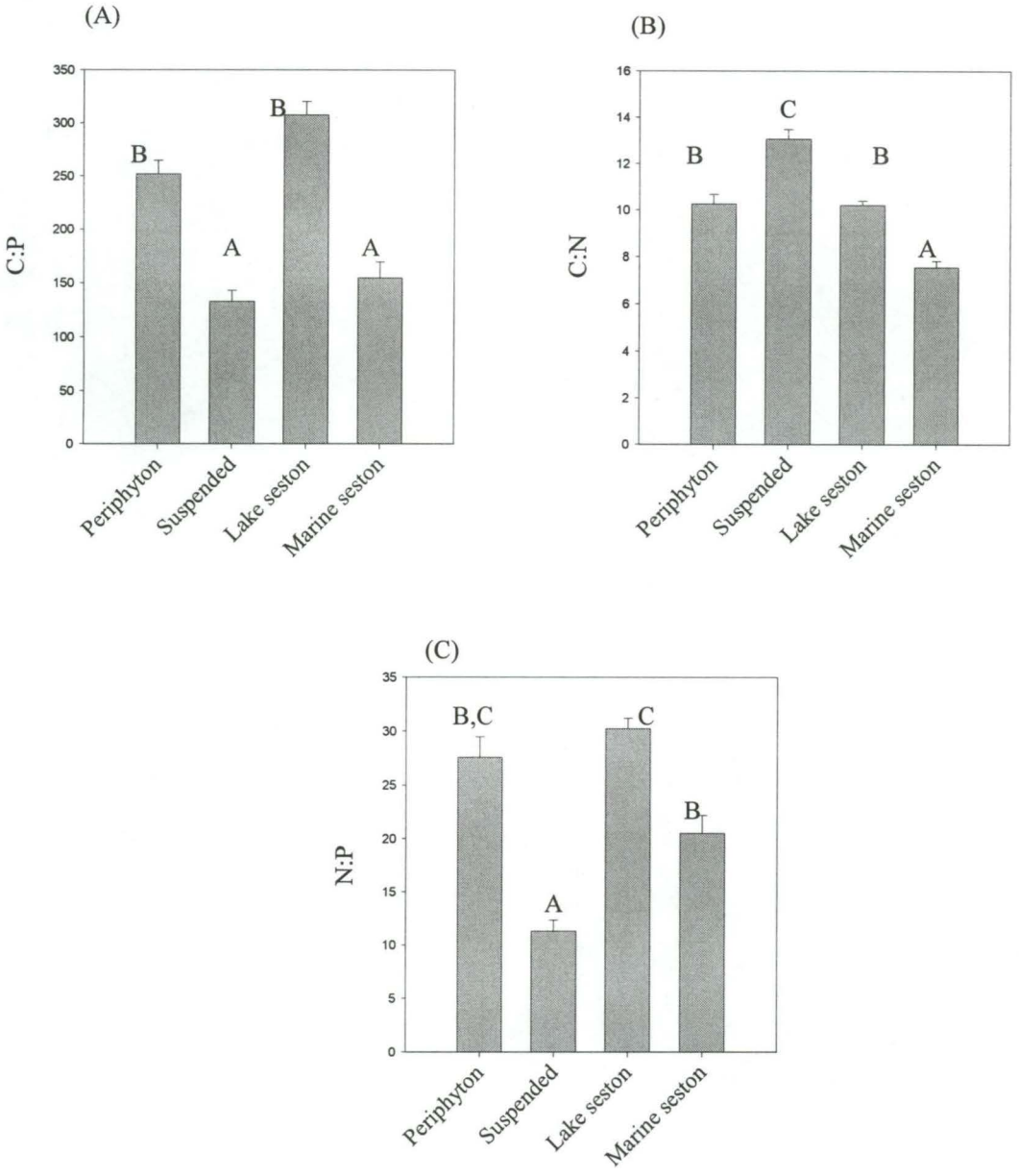

Figure 8. (A) C:P, (B) C:N, and (C) N:P means +/- SE from different aquatic systems. "Periphyton" = Stream periphyton, "Suspended" = Stream suspended matter. Stream data from this study, lake seston data from Elser et al. (2000), marine seston data from Elser and Hassett (1994). Letters indicate groups whose means are not statistically different, Scheffe post-hoc comparison, $P = 0.05$.